# COMPARATIVE ANTIBACTERIAL PROPERTIES *IN VITRO* OF SEVEN OLIVANIC ACID DERIVATIVES: MM 4550, MM 13902, MM 17880, MM 22380, MM 22381, MM 22382 AND MM 22383

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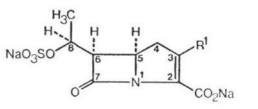
*Streptomyces olivaceus* ATCC 31365 produces a family of novel β-lactam antibiotics collectively referred to as the olivanic acids. Seven such compounds, MM 4550, MM 13902, MM 17880, MM 22380, MM 22381, MM 22382 and MM 22383 have been identified which have the same carbapenem nucleus but with different side chains attached to the nucleus. The compounds with an (8*S*) hydroxyethyl substituent and *cis*-orientated β-lactam protons, MM 22380 and MM 22382, and their sulphate esters, MM 17880 and MM 13902, are potent antibiotics with MIC values against Gram-positive and Gram-negative bacteria in the range 0.1 ~ 3.1 μg/ml. The corresponding (8*S*) hydroxyethyl compounds with *trans*-β-lactam protons, MM 22381 and MM 22383, also have broad-spectrum activity but are rather less potent than the *cis*-compound. In addition to their antibiotic activity, the olivanic acids inhibit a number of β-lactamases and enhance the activity of β-lactams such as amoxycillin against β-lactamase-producing bacteria. Significant differences are observed both in antibacterial activities and in β-lactamase inhibition properties when the olivanic acids are compared with the related thienamycin antibiotics which have (8*R*) rather than (8*S*) stereochemistry and *trans*-β-lactam protons.

The olivanic acids produced by *Streptomyces olivaceus* ATCC 31365 comprise a family of β-lactam antibiotics based on the carbapenem ring system. Previous reports from these laboratories have shown that, depending on the fermentation conditions, this organism produces seven such antibiotics referred to as MM 4550, MM 13902, MM 17880, MM 22380, MM 22381, MM 22382 and MM 22383. Compounds MM 4550, MM 13902 and MM 17880 are sulphated derivatives and are the predominant antibiotics produced by *S. olivaceus* when grown in media containing sodium sulphate[1~4]. The non-sulphated analogues MM 22380, MM 22381, MM 22382 and MM 22383, designated the hydroxy compounds, are produced in sulphate-free media or by mutants of *S. olivaceus* unable to complete the terminal sulphation process.[5,6]

In recent years other reports describing related compounds from various *Streptomyces* spp. have appeared. For example, Merck Sharp and Dohme Research Laboratories have described thienamycin, N-acetylthienamycin and N-acetyldehydrothienamycin from *S. cattleya*[7] and also a group of antibiotics, the epithienamycins A ~ D from *S. flavogriseus*.[8,9] The olivanic acid derivatives MM 22380, MM 22381, MM 22382 and MM 22383 have the same gross structure as the epithienamycins although the exact stereochemistry of the last-named compounds has not yet been published. Very recently another closely related analogue, PS-5, has been reported to be produced by *S. cremeus* subspecies *auratilis*[10,11]. The structural relationships between these compounds are shown in Fig. 1. It can be seen that a major difference between the olivanic acids and thienamycin is the absolute stereochemistry of the chiral centre at C-8 which is (*S*) for the seven olivanic acid compounds but (*R*) for thienamycin. The various reports describe the compounds as potent broad-spectrum antibiotics[4,8,12,13] and show that

Fig. 1. Structures of olivanic acids and related antibiotics.

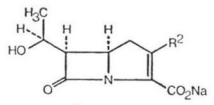
(a) Sulphated olivanic acids (5*R*, 6*R*, 8*S*)



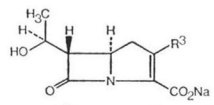
| $R^1$ | Compound |
|---|---|
| $-^{+}SCH=CHNHCOCH_3$ | MM 4550 |
| $-SCH=CHNHCOCH_3$ | MM 13902 |
| $-SCH_2CH_2NHCOCH_3$ | MM 17880 |

(b) Hydroxy olivanic acids

(i) *cis*-compounds(5*R*, 6*R*, 8*S*) (ii) *trans*-compounds (5*R*, 6*S*, 8*S*)



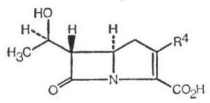
| $R^2$ | Compound |
|---|---|
| $-SCH_2CH_2NHCOCH_3$ | MM 22380 |
| $-SCH=CHNHCOCH_3$ | MM 22382 |



| $R^3$ | Compound |
|---|---|
| $-SCH_2CH_2NHCOCH_3$ | MM 22381 |
| $-SCH=CHNHCOCH_3$ | MM 22383 |

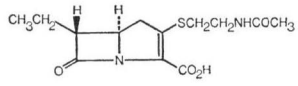
(c) Other related antibiotics

(i) Thienamycin analogues (5*R*, 6*S*, 8*R*)



| $R^4$ | Compound |
|---|---|
| $-SCH_2CH_2NH_2$ | Thienamycin |
| $-SCH_2CH_2NHCOCH_3$ | N-Acetylthienamycin |
| $-SCH=CHNHCOCH_3$ | N-Acetyldehydrothienamycin |

(ii) PS-5



some act also as $\beta$-lactamase inhibitors and are synergistic in combination with other antibiotics[1,4,6,14]. This paper compares the antibacterial and $\beta$-lactamase-inhibitory properties of the seven olivanic acid derivatives and considers also the structure-activity relationships seen between certain of these compounds and the related antibiotics N-acetylthienamycin and thienamycin.

## Materials and Methods

### Compounds

The olivanic acid derivatives were prepared by fermentation of *S. olivaceus* ATCC 31365 as described previously[4,6]. Potassium clavulanate and amoxycillin free acid were Beecham Laboratory Reference Standards, and N-acetylthienamycin and thienamycin were kindly supplied by Dr. J. BIRNBAUM of Merck Sharp and Dohme Research Laboratories.

### Minimum Inhibitory Concentrations (MIC)

For the majority of organisms the compounds were serially diluted in 0.05 ml volumes of Nutrient Broth (Oxoid) using microtitre equipment (Dynatech). TODD-HEWITT Broth (Oxoid) was used for testing against streptococci; Brain Heart Infusion (Oxoid) plus LEVINTAL broth (2: 1) was used for *Haemophilus influenzae*, and SCHAEDLER Broth (Oxoid) for the growth of *Bacteroides fragilis*. All microtitre trays were inoculated with a multipoint inoculator (Denleytech) which delivered 0.001 ml of a 1/10 dilution of an overnight broth culture, an inoculum equivalent to $10^6$ c.f.u./ml. The MIC was determined after incubation at 37°C for 18 hours for aerobes or after 24 hours at 37°C in 95% $H_2$/5% $CO_2$ in a Gaspack Jar (BBL) for *B. fragilis*, as the lowest concentration of antibiotic preventing visible growth.

### 6-hour/18-hour MIC values

Serial dilutions of antibiotic (0.2 ml) and nutrient broth (1.8 ml) were added to each well of a $4 \times 6$ well tissue culture dish (Flow Laboratories) and inoculated with 0.02 ml of a 1/5 dilution of an overnight broth culture (about $10^6$ c.f.u./ml). MIC values were determined after 6-hour and after 18-hour incubations at 37°C.

### Synergism Studies

Serial dilutions of amoxycillin were added to 18 ml volumes of Blood Agar Base (Oxoid) together with a constant concentration of the test inhibitor at 1/5 or 1/10 MIC value and poured into Petri dishes. Control plates without test inhibitor were also prepared. The plates were dried at 37°C for 30 minutes and inoculated with 0.001 ml of an undiluted overnight broth culture of the test strain by means of a replicating device, giving an inoculum of about $10^6$ c.f.u. MIC values were determined after 18 hours at 37°C and synergism was said to have occurred when the inhibitory concentration of amoxycillin in the presence of inhibitor was reduced at least four-fold compared with that of amoxycillin alone.

## Results

### Antibacterial Activities of Olivanic Acid Derivatives

The antibacterial spectra of the seven olivanic acid analogues are illustrated in Table 1; each MIC value represents the median obtained against ten clinical isolates. It can be seen that the sulphated deri-

Table 1. Antibacterial spectrum of seven olivanic acid derivatives.

| Organism* | Median MIC ($\mu$g/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MM 4550 | MM 13902 | MM 17880 | MM 22380 | MM 22381 | MM 22382 | MM 22383 |
| *Escherichia coli* $Amp^S$ | 12.5 | 0.2 | 0.2 | 0.2 | 6.2 | 0.2 | 12.5 |
| *Escherichia coli* $Amp^R$ $R_{TEM}$ | 25 | 1.6 | 1.6 | 25 | 6.2 | 25 | 12.5 |
| *Klebsiella aerogenes* $Cep^S$ | 12.5 | 0.4 | 0.4 | 0.8 | 6.2 | 0.4 | 6.2 |
| *Klebsiella aerogenes* $Cep^R$ $R_{TEM}$ | 50 | 3.1 | 3.1 | 50 | 6.2 | 100 | 12.5 |
| *Proteus mirabilis* $Amp^S$ | 12.5 | 0.2 | 0.4 | 0.8 | 12.5 | 0.2 | 12.5 |
| *Proteus mirabilis* $Amp^R$ | 12.5 | 0.2 | 0.4 | 0.8 | 12.5 | 0.2 | 12.5 |
| *Proteus morganii* | 25 | 0.4 | 0.8 | 1.6 | 12.5 | 0.8 | 25 |
| *Proteus rettgeri* | 25 | 0.4 | 0.8 | 3.1 | 12.5 | 1.6 | 12.5 |
| *Proteus vulgaris* | 12.5 | 0.4 | 0.8 | 1.6 | 6.2 | 0.8 | 6.2 |
| *Enterobacter aerogenes* | 25 | 3.1 | 3.1 | 1.6 | 6.2 | 1.6 | 12.5 |
| *Enterobacter cloacae* | 100 | 12.5 | 6.2 | 3.1 | 12.5 | 3.1 | 25 |
| *Serratia marcescens* | 25 | 3.1 | 3.1 | 3.1 | 12.5 | 3.1 | 12.5 |
| *Pseudomonas aeruginosa* | >100 | 25~50 | 100 | >100 | >100 | >100 | >100 |
| *Haemophilus influenzae* $Amp^S$ | 6.2 | 0.1 | 0.2 | 0.2 | 6.2 | 0.1 | 6.2 |
| *Haemophilus influenzae* $Amp^R$ $R_{TEM}$ | 6.2 | 0.1 | 0.2 | 1.0 | 6.2 | 0.5 | 6.2 |
| *Bacteroides fragilis* | 3.1 | 0.4 | 0.4 | 0.4 | 3.1 | 0.4 | 3.1 |
| *Staphylococcus aureus* $Amp^S$ | 25 | 1.6 | 1.6 | 0.4 | 3.1 | 0.4 | 6.2 |
| *Staphylococcus aureus* $Amp^R$ | 50 | 1.6 | 1.6 | 0.8 | 3.1 | 0.4 | 6.2 |
| *Staphylococcus aureus* $Meth^R$ | 100 | 12.5 | 12.5 | 6.2 | 100 | 6.2 | 100 |
| *Streptococcus pyogenes* | 6.2 | 0.2 | 0.1 | 0.1 | 0.8 | 0.05 | 1.6 |
| *Streptococcus faecalis* | 50 | 6.2 | 6.2 | 1.6 | 25 | 1.6 | 50 |

* $Amp^{S/R}$; $Cep^{S/R}$: denotes sensitivity or resistance to ampicillin or cephalothin
$Meth^R$: methicillin-resistant strain
$R_{TEM}$: Strain producing $R_{TEM}$ $\beta$-lactamase.

vatives, MM 13902 and MM 17880, and the hydroxy compounds with *cis*-orientated $\beta$-lactam protons (*i.e.* 5*R*, 6*R*), MM 22380 and MM 22382, are potent broad-spectrum antibiotics which inhibited the majority of organisms at concentrations as low as 0.1~3.1 $\mu$g/ml. The compounds were active against *Escherichia coli*, *Klebsiella aerogenes*, *Proteus mirabilis*, Streptococci (including Enterococci) and penicillin-sensitive and resistant strains of *Staphylococcus aureus* or *Haemophilus influenzae*. In addition, the olivanic acids showed activity against organisms that are often resistant to other $\beta$-lactams such as, indole-positive *Proteus, Enterobacter* spp, *Serratia marcescens* and the anaerobe *Bacteroides fragilis*. However, as a rule the compounds had relatively poor activity against *Pseudomonas aeruginosa* and methicillin-resistant strains of *Staphylococcus aureus*.

Analogue MM 4550, which is a sulphoxide of MM 13902, and the (8*S*)-hydroxy compounds with *trans*-orientated $\beta$-lactam protons (*i.e.* 5*R*, 6*S*), MM 22381 and MM 22383, show antibacterial spectra similar to those of the previously described (5*R*, 6*R*) analogues but were rather less potent with MIC values of between 3.1~25 $\mu$g/ml against most strains.

The relatively poor activity of MM 4550 compared to MM 13902 as shown in Table 1 reflects a difference in the chemical stabilities of the two compounds rather than an inherent difference in their antibacterial potencies. For example, under the conditions of a normal 18-hour broth MIC test, the half-

Table 2. Antibacterial activity of MM 4550 and MM 13902 in MIC tests read at 6 and 18 hours.

| Organism | MIC ($\mu$g/ml) | | | |
|---|---|---|---|---|
| | MM 4550 | | MM 13902 | |
| | 6 hr. | 18 hr. | 6 hr. | 18 hr. |
| *E. coli* 0111 | 0.3 | 10 | 0.1 | 0.3 |
| *E. coli* JT39 ($R_{TEM}$) | 0.3 | 10 | 0.1 | 0.6 |
| *K. aerogenes* A | 0.3 | 2.5 | 0.05 | 0.1 |
| *P. aeruginosa* NCTC 10662 | 50 | >100 | 6.2 | 100 |
| *S. aureus* Russell | 1.2 | 20 | 0.6 | 1.2 |

life of MM 13902 at 37°C was about 12 hours, whereas that of MM 4550 was only 2 hours. When MIC values were determined after 6-hour incubation, the activity of MM 4550 against most organisms was only two to four-fold less than that of MM 13902 (Table 2). However, between 6 and 18 hours the activity of MM 13902 showed only a slight reduction whereas that of MM 4550 decreased considerably. The results in Table 2 show also that MM 13902 was rather more active against *P. aeruginosa* when MIC values were determined at 6 hours than at 18 hours, a property not seen with MM 4550. The poorer activity of MM 22381 and MM 22383 is not due to a similar instability in the test system.

## Structure-Activity Relationships between Certain Olivanic Acid and Thienamycin Derivatives

The effect on antibacterial activity of the substituent at position C-8 or the relative stereochemistry at positions C-6 and C-8 is illustrated in Table 3 with reference to four compounds, MM 17880, MM 22380, MM 22381 and N-acetylthienamycin. Each of these compounds has a 2-acetamidoethylthio side chain at position C-3. Compound MM 22380 has an (*S*)-hydroxyethyl side chain at position C-6 and *cis*-protons (5*R*, 6*R*) on the $\beta$-lactam. It is a potent broad-spectrum antibiotic active against a number of Gram-positive and Gram-negative bacteria. A characteristic feature of MM 22380 is the marked reduction in activity seen against strains of *E. coli* or *K. aerogenes* that produce $R_{TEM}$ $\beta$-lactamase compared with that against strains not producing the enzyme. This difference in activities can

Table 3. Comparative antibacterial activities of MM 17880, MM 22380, MM 22381, N-acetylthienamycin and thienamycin.

| Organism | MIC value ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|
| | MM 17880 *cis*-(6*R*, 8*S*) (sulphate) | MM 22380 *cis*-(6*R*, 8*S*) (hydroxy) | MM 22381 *trans*-(6*S*, 8*S*) (hydroxy) | N-Acetyl thienamycin *trans* (6*S*, 8*R*) (hydroxy) | Thienamycin (deacetyl derivative) |
| *Escherichia coli* 0111 | 0.2 | 0.2 | 6.2 | 0.8 | 0.2 |
| *Escherichia coli* JT39 ($R_{TEM}$) | 1.6 | 25 | 6.2 | 0.8 | 0.4 |
| *Klebsiella aerogenes* A | 0.4 | 0.8 | 6.2 | 0.4 | 0.4 |
| *Klebsiella aerogenes* VA2 ($R_{TEM}$) | 3.1 | 50 | 6.2 | 0.8 | 0.4 |
| *Proteus mirabilis* 977 | 0.4 | 0.8 | 12.5 | 3.1 | 3.1 |
| *Proteus rettgeri* WM16 | 0.8 | 1.6 | 12.5 | 3.1 | 3.1 |
| *Enterobacter aerogenes* T728 | 3.1 | 1.6 | 6.2 | 3.1 | 1.6 |
| *Serratia marcescens* US20 | 3.1 | 3.1 | 12.5 | 3.1 | 1.6 |
| *Pseudomonas aeruginosa* NCTC 10662 | >100 | >100 | >100 | 25 | 3.1 |
| *Bacteroides fragilis* BC-16 | 0.4 | 0.4 | 3.1 | NT | 0.4 |
| *Staphylococcus aureus* Russell | 1.6 | 0.4 | 3.1 | 0.2 | 0.04 |
| *Streptococcus faecalis* I | 6.2 | 1.6 | 25 | 6.2 | 1.6 |
| *Streptococcus pyogenes* CN10 | 0.1 | 0.05 | 0.8 | 0.05 | 0.01 |

Table 4. Synergism between amoxycillin and olivanic acid derivatives compared with amoxycillin and clavulanic acid against β-lactamase-producing bacteria.

| Combinations (μg/ml) | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | *E. coli* JT 39 | *K. aerogenes* A | *P. mirabilis* 889 | *E. cloacae* N1 | *S. aureus* Russell |
| Amoxycillin alone | >1,000 | 250 | >1,000 | 500 | 250 |
| 〃 +MM 4550 (1.0) | 250† | 5.0† | 100† | 500 | 1.0† |
| 〃 +MM 13902 (0.05) | >1,000 | 100 | >1,000 | 500 | 5.0† |
| 〃 +MM 17880 (0.05) | 1,000 | 50† | >1,000 | 500 | 5.0† |
| 〃 +MM 22380 (0.1) | >1,000 | 250 | >1,000 | 500 | 5.0† |
| 〃 +MM 22381 (0.5) | 500† | 100 | >1,000 | 500 | 250 |
| 〃 +MM 22382 (0.1) | >1,000 | 250 | >1,000 | 500 | 1.0† |
| 〃 +MM 22383 (0.5) | 250† | 25† | >1,000 | 500 | 100 |
| 〃 +Clavulanic acid (1.0) | 50† | 5.0† | 500† | 500 | 1.0† |
| 〃 +Clavulanic acid (5.0) | 12.5† | 1.0† | 25† | 500 | 0.2† |

† Denotes synergy

be correlated with an instability to the isolated $R_{TEM}$ β-lactamase (S. Box, personal communication). The sulphate ester of MM 22380, compound MM 17880, is likewise a potent broad-spectrum antibiotic, which is slightly less active than MM 22380 against Gram-positive cocci but considerably more effective against strains of *E. coli* or *K. aerogenes* mediating $R_{TEM}$ β-lactamase. Biochemical studies have shown that MM 17880 is stable to isolated $R_{TEM}$ β-lactamase (S. Box, personal communication).

Compound MM 22381 is an isomer of MM 22380 that retains the (*S*)-hydroxyethyl side chain but has *trans*-protons on the β-lactam ring (*i.e.* 5*R*, 6*S*). This change considerably reduces the antibacterial potency of the compound compared to the (5*R*, 6*R*) isomer but imparts stability to $R_{TEM}$ β-lactamase such that the antibiotic is as active against plasmid-carrying strains of *E. coli* or *K. aerogenes* as against non-plasmid-carrying strains. N-Acetylthienamycin can likewise be considered as an isomer of MM 22381 as it has the same stereochemistry at C-6 but the hydroxyethyl side chain is *R* rather than *S*. It can be seen from Table 3 that N-acetylthienamycin has much improved antibacterial potency compared to MM 22381, being similar to that of the (6*R*, 8*S*) isomer MM 22380, with slightly reduced activity against *Proteus* spp., and retains the stability of the (6*S*, 8*S*) compound towards $R_{TEM}$ β-lactamase. All these compounds have at best only a moderate level of activity against *P. aeruginosa* (MIC 25~>100 μg/ml). However, deacetylation of N-acetylthienamycin to the free amino analogue, namely thienamycin, improves the level of activity against *P. aeruginosa* and against Gram-positive cocci whilst retaining the overall potency and β-lactamase stability of N-acetylthienamycin against other Gram-negative bacteria (Table 3).

## Synergism between Olivanic Acids and Amoxycillin against β-Lactamase-Producing Organisms

The effect of sub-inhibitory concentrations of the olivanic acids on the activity of amoxycillin against β-lactamase-producing bacteria is shown in Table 4. Comparable data are shown also for clavulanic acid. It can be seen that synergy occurred against *S. aureus* with most of the olivanic acids, other than MM 22381 and MM 22383. For example, concentrations of 0.05~1.0 μg/ml reduced the MIC values of amoxycillin from 250 μg/ml to 1.0~5.0 μg/ml and produced synergistic effects similar to that seen with amoxycillin and clavulanic acid (1.0 μg/ml). Against the Gram-negative bacteria, *E. coli, K. aerogenes*

and *P. mirabilis* only MM 4550 resulted in synergy comparable to that seen with clavulanic acid and none of the compounds enhanced the activity of amoxycillin against a strain of *E. cloacae*. However, clavulanic acid has only poor antibacterial activity and it can be seen from Table 4 that increasing the concentration from 1.0~5.0 μg/ml improves the synergy obtained with amoxycillin. In contrast the olivanic acids are potent antibacterial agents, and similar increases in concentration are often inhibitory in their own right and invalidate any synergy experiments.

## Discussion

The data presented in this paper show that all seven olivanic acid derivatives and the two thienamycin analogues tested are broad-spectrum compounds although they vary in β-lactamase stability and in their intrinsic level of antibacterial activity. For example, in the (8*S*) series the hydroxy compounds with *cis*-β-lactam protons, MM 22380 and MM 22382, are potent antibiotics but lack stability to $R_{TEM}$ β-lactamase and hence show poor activity against the strains of *E. coli* and *K. aerogenes* that produce this enzyme. The sulphate esters of these compounds, MM 13902 and MM 17880, are likewise potent antibiotics but in addition are stable to $R_{TEM}$ β-lactamase and show considerably improved activity against the strains resistant to MM 22380 and MM 22382. In contrast the (8*S*) hydroxy compounds with *trans*-β-lactam protons, MM 22381 and MM 22383 are considerably less potent than their *cis*-hydroxy counterparts although are stable to $R_{TEM}$ β-lactamase. Thienamycin and N-acetylthienamycin, like MM 22381 or MM 22383, have *trans*-β-lactam protons, but differ in their stereochemistry at C-8 being (8*R*) rather than (8*S*). This change improves the antibacterial potency without affecting the stability to $R_{TEM}$ β-lactamase found in the (8*S*) *trans*-compounds. Thus it would seem that compounds with *trans*-β-lactam protons and (8*R*) stereochemistry or those with *cis*-β-lactam protons and (8*S*) stereochemistry are the most potent antibiotics although the (8*S*) hydroxy derivatives show instability to $R_{TEM}$ β-lactamase.

As well as being potent antibacterial agents, the olivanic acids also inhibit a number of β-lactamases. A previous report showed the (8*S*) sulphated compounds, MM 4550, MM 13902 and MM 17880 to be very effective inhibitors of isolated β-lactamase preparations from *E. coli* ($R_{TEM}$), *K. aerogenes*, *P. mirabilis*, *E. cloacae* and *S. aureus*[4]. The (8*S*) hydroxy compounds MM 22380, MM 22381, MM 22382 and MM 22383 were shown later to be less active than their sulphate esters but to have a similar inhibitory spectrum[6]. Interestingly compounds with (8*R*) stereochemistry, such as thienamycin and N-acetylthienamycin, which are potent antibiotics had only poor β-lactamase inhibitory properties (C. READING, personal communication).

In synergy experiments the olivanic acids in combination with amoxycillin produced synergism comparable to that seen with clavulanic acid and amoxycillin against *S. aureus*, with the exception of MM 22381 and MM 22383 which are poor inhibitors of staphylococcal β-lactamase[6]. However, the extent of synergy against Gram-negative bacteria was less than might have been expected from the β-lactamase inhibitory activities of the compounds. Only MM 4550 was effective in combination with amoxycillin being as effective as amoxycillin plus clavulanic acid. Likewise, none of the olivanic acids enhanced the activity of amoxycillin against *E. cloacae* although all were capable of inhibiting the isolated enzyme[4,6]. The reasons for these discrepancies are currently being investigated although, as mentioned previously, the potent antibacterial activity of these compounds complicates the interpretation of the synergy experiments.

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