MUTUAL PRO-DRUGS OF THE OLIVANIC ACIDS AND RENAL DIPEPTIDASE INHIBITORS

M. J. BASKER, S. COULTON* and R. SOUTHGATE

Beecham Pharmaceuticals, Chemotherapeutic Research Centre,
Brockham Park, Betchworth, Surrey, RH3 7AJ England

(Received for publication October 15, 1984)

The carbapenem antibiotics, which include the olivanic acids and thienamycin, possess potent broad spectrum antibacterial activity. They are however extensively metabolized by the renal dipeptidase enzyme, dehydropeptidase I. As a result of this degradation, only low urinary recoveries of antibiotic are obtained *in vivo*.

The preparation of mutual pro-drugs of the olivanic acids and an inhibitor of the renal dipeptidase enzyme is described. MM 22382 and MM 13902 have been combined with *Z*-2-isovaleramidobut-2-enoic acid as double esters of formaldehyde hydrate. Administration of these linked esters to mice results in improved urinary recoveries of antibiotic.

Since the isolation of the olivanic acids[1,2] and thienamycin[3] from soil micro-organisms, a multitude of structurally related natural products have been reported[4~7]. In general, these streptomycete metabolites, collectively known as the carbapenem antibiotics, exhibit a high degree of activity ag inst a broad range of Gram-positive and Gram-negative bacteria.

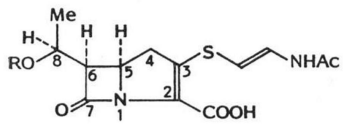
Although most carbapenems display remarkable stability towards bacterial β-lactamases, it has recently been found that they are extensively metabolized by the renal dipeptidase enzyme, dehydropeptidase I (DHP-I)[8]. Consequently, these compounds give only low urinary recoveries *in vivo*.

The metabolic instability of thienamycin and MK 0787 (*N*-formimidoylthienamycin) has been overcome by co-administration with an inhibitor of the renal dipeptidase enzyme. Various 3-substituted *Z*-2-acylaminopropenoic acids have been shown to protect the antibiotic by preventing the enzymatic hydrolysis of the β-lactam ring[9,10]. Thus, use of such combinations has resulted in improved urinary recoveries of the antibiotic in both human and animal species[11,12].

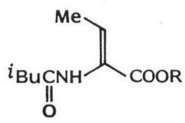
The concept of the "mutual pro-drug" is now well documented[13]. A recent report from the Leo group described the combination of ampicillin with the β-lactamase inhibitor, penicillanic acid sulphone, in the form of a single molecule[14]. The two compounds were linked *via* their carboxyl groups as a double ester of formaldehyde hydrate. Upon administration, esterase hydrolysis provides the individual components in an equimolar ratio. We have applied the same principle to the combination of carbapenem antibiotics and renal dipeptidase inhibitors. The present paper describes the preparation and biological evaluation of mutual pro-drugs derived from *Z*-2-isovaleramidobut-2-enoic acid (**3**) and the olivanic acids, MM 22382 (**1**) and MM 13902 (**2**).

Chemistry

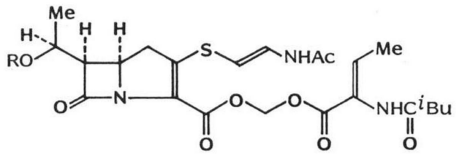
The linked ester (**6**) was prepared by reacting the sodium salt of MM 22382 (**1**) with the iodomethyl ester of *Z*-2-isovaleramidobut-2-enoic acid (**5**) in *N,N*-dimethylformamide. Similarly, treatment of the disodium salt of MM 13902 (**2**) with the iodo-derivative (**5**) afforded the sodium salt of double ester (**7**). The iodomethyl ester (**5**) was prepared from the carboxylic acid (**3**), *via* the chloromethyl ester (**4**). Reaction of **3** with chloromethyl chlorosulfate, cetyl-dimethylbenzylammonium chloride and



MM 22382 (**1**) R = H
MM 13902 (**2**) R = SO_3H



3 R = H
4 R = $COOCH_2Cl$
5 R = $COOCH_2I$



6 R = H
7 R = SO_3H

sodium bicarbonate in dichloromethane - water furnished the chloromethyl ester (**4**) as a crystalline solid. Subsequent treatment of **4** with sodium iodide in refluxing acetone provided the iodomethyl ester (**5**).

Biology

The *in vitro* antibacterial activities of the mutual pro-drugs, **6** and **7**, and the corresponding parent antibiotics, MM 22382 and MM 13902 respectively, are presented in Table 1. In general, the mutual pro-drugs displayed antibacterial activity which was four to eight-fold less than that of the parent antibiotic, suggesting partial hydrolysis of the ester linkage under the test conditions.

MM 22382 and MM 13902 are readily hydrolysed by the renal dipeptidase enzyme and give very low urinary recoveries (0.3% and 1.3% respectively) in the mouse (see Table 2). When the compounds were dosed in the form of the mutual pro-drug, the urinary recoveries were increased approximately six-fold. Thus, 1.8% of MM 22382 was recovered in the urine of mice dosed with the linked ester (**6**) and 8% of MM 13902 was recovered from animals which received **7**. These values were only marginally lower than those obtained when the antibiotic and renal dipeptidase inhibitor were co-administered as individual components.

In conclusion, the results of this study would indicate that the combination of the carbapenem antibiotic with a renal dipeptidase inhibitor, in the form of a mutual pro-drug, is a convenient and effective method of improving the urinary recovery of the antibiotic *in vivo*.

Experimental

MM 13902 and MM 22382 were prepared by fermentation of *Streptomyces olivaceus* ATCC 31365 as described previously[1,2]. *Z*-2-Isovaleramidobut-2-enoic acid (**3**) was prepared by the method described in reference 9 and chloromethyl chlorosulfate was prepared by the method described in reference 15.

Minimum Inhibitory Concentrations (MIC)

The compounds were serially diluted in 0.05 ml volumes of Nutrient Broth No. 2 (Oxoid) using microtitre equipment (Dynatech). 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 of the test organism, an inoculum equivalent to 10^6 cfu/ml. The MIC was determined after incubation at 37°C for 18 hours as the lowest concentration of antibiotic preventing visible microbial growth.

Urinary Recoveries in Mice

For each compound or combination of compounds, three groups of five mice were given 1 ml water orally and dosed by subcutaneous injection. Urine was collected over 0~1 hour, 1~2 hours and 2~4 hours periods. Antibiotic concentrations in urine samples were determined by microbiological assay using *Bacillus subtilis* against standards of MM 13902 or MM 22382 prepared in 0.05 M phosphate buffer

Table 1. Comparative *in vitro* antibacterial activities of the linked esters, **6** and **7** and the corresponding parent antibiotics, MM 22382 and MM 13902, respectively.

Organism	MIC (μg/ml)			
	6	MM 22382	**7**	MM 13902
Enterobacter cloacae N1	6.2	1.6	3.1	0.4
Escherichia coli 0111	0.8	0.2	3.1	0.4
E. coli JT39 (amp^R)	25	3.1	1.6	0.2
Klebsiella pneumoniae A	6.2	0.8	0.4	0.4
Proteus mirabilis 977	3.1	0.4	3.1	0.4
P. rettgeri WM16	6.2	6.2	0.8	0.8
P. vulgaris W091	12.5	3.1	1.6	0.4
Pseudomonas aeruginosa A	>100	>50	100	100
Serratia marcescens US20	6.2	3.1	6.2	0.8
Staphylococcus aureus Oxford	0.4	0.4	1.6	1.6
S. aureus Russell (amp^R)	0.8	0.4	3.1	3.1
Streptococcus faecalis I	3.1	0.8	50	12.5

amp^R: Denotes resistance to ampicillin.

Table 2. Percentage urinary recoveries of MM 22382 and MM 13902 when administered subcutaneously to mice i) alone, ii) as a combination with the renal dipeptidase inhibitor (**3**), iii) as the linked esters (**6**) and (**7**).

	Compound	Dose	% urinary recovery 0~4 hours
i	MM 22382 alone	50 mg/kg	0.3
ii	MM 22382+**3**	50 mg/kg MM 22382+27.7 mg/kg (**3**) (equimolar ratio)	2.4
iii	**6**	10 mg/kg (equivalent to 6.6 mg/kg MM 22382)	1.8
i	MM 13902 alone	50 mg/kg	1.3
ii	MM 13902+**3**	50 mg/kg MM 13902+50 mg/kg **3**	8.5
iii	**7**	10 mg/kg (equivalent to 7.2 mg/kg MM 13902)	8.0

(pH 7.0).

Chemistry: General

MP were determined on a Kofler hot-stage apparatus and are uncorrected. UV spectra were recorded on a Perkin-Elmer 554 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 983 machine. ^{1}H NMR spectra were recorded at 90 MHz on a Perkin-Elmer R32 and at 250 MHz on a Bruker WM 250 instrument with tetramethylsilane as internal standard. Mass spectra were determined on a VG 70-70 or a VG ZAB instrument. The purity of all compounds was tested by TLC on Merck pre-coated silica gel F_{254} plates. Preparative chromatography was carried out on columns of Merck silica gel 60 (1 : 1 mixture of finer than 230 mesh and 230~400 mesh ASTM) using the slightly increased pressure provided by a Medcalf Hy-flo pump. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Chloromethyl *Z*-2-Isovaleramidobut-2-enoate (**4**)

Z-2-Isovaleramidobut-2-enoic acid (8.9 g), sodium bicarbonate (8.08 g) and cetyl dimethylbenzylammonium chloride (1.90 g) were suspended in dichloromethane - water (1 : 1, 500 ml). To the rapidly stirred suspension was added dropwise a solution of chloromethyl chlorosulfate (9.5 g) in dichloromethane (25 ml). Stirring was continued for 15 minutes at room temperature. The organic phase was then separated, washed with water, saturated sodium chloride solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent at reduced pressure gave the crude product, which was purified by column chromatography over silica gel (100 g). Elution with a gradient of 25~50% ethyl acetate - hexane afforded the pure chloromethyl ester (**4**) as a white solid (1.89 g, 17%) after trituration with

hexane; MP 65~66°C; IR ν_{max} (KBr) cm^{-1} 3278, 1740, 1648 and 1513; ^1H NMR ($CDCl_3$) δ ppm 1.02 (6H, d, J=6.5 Hz, $(H_3C)_2CH$), 1.83 (3H, d, J=6.5 Hz, $H_3CCH=C$), 2.05~2.30 (3H, m, $(H_3C)_2CHCH_2CO$), 5.79 (2H, s, CO_2CH_2Cl), 6.84 (1H, broad s, NH), 6.94 (1H, q, J=6.5 Hz, $H_3CCH=C$). (Found: C 51.6, H 6.75, N 6.1%; M^+, 233.0816. Calcd. for $C_{10}H_{16}NO_3Cl$: C 51.4, H 6.9, N 6.0%; MW 233.0818).

Iodomethyl *Z*-2-Isovaleramidobut-2-enoate (**5**)

The chloromethyl ester (**4**) (1.0 g) and sodium iodide (1.278 g) were dissolved in acetone (100 ml). The solution was heated to reflux for 6 hours. After cooling, the solvent was evaporated at reduced pressure and the residue partitioned between ethyl acetate and water. The organic solution was washed with dilute sodium thiosulfate solution, water, saturated sodium chloride solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent at reduced pressure furnished the crude product which was purified by silica gel column chromatography (30 g). Elution with a gradient of 10~50% ethyl acetate - hexane provided the pure iodomethyl ester (**5**) as a white solid (0.63 g, 45%) after trituration with hexane; MP 93~96°C; IR ν_{max} (KBr) cm^{-1} 3256, 1744, 1658 and 1520; ^1H NMR ($CDCl_3$) δ ppm 0.98 (6H, d, J=6 Hz, $(H_3C)_2CH$), 1.78 (3H, d, J=7 Hz, $H_3CCH=C$), 2.0~2.3 (3H, m, $(H_3C)_2CHCH_2CO$), 5.92 (2H, s, CO_2CH_2I), 6.5~6.95 (2H, q+broad resonance, $H_3CCH=C+NH$). (Found: C 36.95, H 5.1, N 4.35%; M^+ 325.0181. Calcd for $C_{10}H_{16}NO_3I$: C 36.95, H 4.95, N 4.3%; MW 325.0177).

Z-2-Isovaleramidobut-2-enoyloxymethyl (5*R*,6*R*)-3-[(*E*)-2-Acetamidovinylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**6**)

The sodium salt of MM 22382 (**1**) (0.222 g) was dissolved in dry *N,N*-dimethylformamide (5 ml) and stirred at room temperature for 2 hours with the iodomethyl ester (**5**) (0.075 g). The reaction mixture was then partitioned between ethyl acetate and water. The organic solution was washed with dilute sodium thiosulfate solution, water, saturated sodium chloride solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent at reduced pressure afforded the crude product, which was purified by silica gel column chromatography (5 g). Elution of the column with 10% ethanol - chloroform gave the pure diester (**6**) as a pale yellow solid (0.024 g, 20%) after trituration with diethyl ether; MP 136~140°C; $[\alpha]_D^{20}$ −74° (*c* 0.5, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε) 325 (12,880), 227 (17,490); IR ν_{max} (KBr) cm^{-1} 3357, 1773, 1702, 1668, 1658, 1621 and 1507; ^1H NMR ($CDCl_3$) δ ppm 1.01 (6H, d, J=6.5 Hz, $(H_3C)_2CH$), 1.34 (3H, d, J=6.5 Hz, H_3CCH), 1.79 (3H, d, J=7.0 Hz, $H_3CCH=C$), 2.08 (3H, s, $COCH_3$), 2.10~2.25 (3H, m, $(H_3C)_2CHCH_2CO$), 3.06 (1H, dd, J=10 Hz and 18.5 Hz, 4-H_a), 3.36 (1H, dd, J=9 Hz and 18.5 Hz, 4-H_b), 3.54 (1H, dd, J=5.5 Hz and 9 Hz, 6-H), 4.03~4.17 (1H, m, 8-H), 4.17~4.30 (1H, m, 5-H), 5.82 (1H, d, J=13.5 Hz, $SCH=C$), 5.92 (2H, ABq, J=5.5 Hz, CO_2CH_2O), 6.93 (1H, q, J=7 Hz, $H_3CCH=C$), 7.21 (1H, dd, J=10 Hz and 13.5 Hz, $C=CHNH$), 7.36 (1H, s, NH), 8.91 (1H, d, J=10 Hz, NH). (Found: C 54.2, H 6.2, N 8.05%. Calcd for $C_{23}H_{31}N_3O_8S$: C 54.2, H 6.15, N 8.25%).

Sodium Salt of *Z*-2-Isovaleramidobut-2-enoyloxymethyl (5*R*,6*R*)-3-[(*E*)-2-Acetamidovinylthio]-6-[(*S*)-1-hydroxysulfonyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**7**)

A solution of the disodium salt of MM 13902 (**2**) (0.265 g) and the iodomethyl ester (**5**) (0.075 g) in dry *N,N*-dimethylformamide (5 ml) was stirred at room temperature for 90 minutes. The solvent was then evaporated to small volume at reduced pressure and the residue applied to a column of silica gel (6 g). Elution with a gradient of 0~30% ethanol - chloroform yielded the crude product **7** as a white solid. This solid was rechromatographed over silica gel (3 g), eluting with a gradient of 0~20% ethanol-chloroform. The pure ester **7** was obtained as a white solid (0.067 g, 48%); MP 173~176°C (dec) (acetone - ether); $[\alpha]_D^{20}$ −90° (*c* 0.5, H_2O); UV $\lambda_{max}^{H_2O}$ nm (ε) 327 (12,149), 225 (18,755); IR ν_{max} (KBr) cm^{-1} 3432, 1752, 1662, 1648, 1622 and 1517; ^1H NMR (DMF-d_7) δ ppm 0.96 (6H, d, J=6 Hz, $(H_3C)_2CH$), 1.44 (3H, d, J=6 Hz, H_3CCH), 1.77 (3H, d, J=6.5 Hz, $H_3CCH=C$), 2.02 (3H, s, $COCH_3$), 2.02~2.25 (3H, m, $(H_3C)_2CHCH_2CO$), 3.05 (1H, dd, J=10 Hz and 19 Hz, 4-H_a), 3.71 (1H, dd, J=5.5 Hz and 10.5 Hz, 6-H), 3,86 (1H, dd, J=8.5 Hz and 10 Hz, 4-H_b), 4.29 (1H, dt, J=5.5 Hz and 9 Hz, 5-H), 4.53 (1H, m, 8-H), 5.94 (3H, d+ABq, J=14.5 Hz and 5 Hz, $SCH=C$ and CO_2CH_2O), 6.60 (1H,

q, J=6.5 Hz, H_3CCH=C), 7.21 (1H, dd, J=10.5 Hz and 14.5 Hz, C=CHNH), 9.23 (1H, s, NH), 10.69 (1H, d, J=10.5 Hz, NH). (Found: C 42.65, H 5.05, N 6.2%. Calcd for $C_{23}H_{30}N_3O_{11}S_2Na \cdot 2H_2O$: C 42.65, H 5.3, N 6.5%).

Acknowledgments

We wish to thank Mrs. E. A. PRESTAGE, Mrs. G. M. SMITH and Mrs. A. V. EXELL for performing the biological studies, Mr. J. W. TYLER and Mr. E. A. CUTMORE for spectroscopic data and Mr. G. M. J. POWELL for microanalytical data. We are also indebted to Dr. D. F. CORBETT for his advice and interest during this work.

References

1) HOOD, J. D.; S. J. BOX & M. S. VERRALL: Olivanic acids, a family of β-lactam antibiotics with β-lactamase inhibitory properties produced by *Streptomyces* species. II. Isolation and characterisation of the olivanic acids MM 4550, MM 13902 and MM 17880 from *Streptomyces olivaceus*. J. Antibiotics 32: 295~304, 1979
2) BOX, S. J.; J. D. HOOD & S. R. SPEAR: Four further antibiotics related to olivanic acid produced by *Streptomyces olivaceus*: Fermentation, isolation, characterisation and biosynthetic studies. J. Antibiotics 32: 1239~1247, 1979
3) KAHAN, J. S.; F. M. KAHAN, R. GOEGELMAN, S. A. CURRIE, M. JACKSON, E. O. STAPLEY, T. W. MILLER, A. K. MILLER, D. HENDLIN, S. MOCHALES, S. HERNANDEZ, H. B. WOODRUFF & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J. Antibiotics 32: 1~12, 1979
4) YAMAMOTO, K.; T. YOSHIOKA, Y. KATO, N. SHIBAMOTO, K. OKAMURA, Y. SHIMAUCHI & T. ISHIKURA: Structure and stereochemistry of antibiotic PS-5. J. Antibiotics 33: 796~803, 1980
5) NAKAYAMA, M.; A. IWASAKI, S. KIMURA, T. MIZOGUCHI, S. TANABE, A. MURAKAMI, I. WATANABE, M. OKUCHI, H. ITOH, Y. SAINO, F. KOBAYASHI & T. MORI: Carpetimycins A and B, new β-lactam antibiotics. J. Antibiotics 33: 1388~1390, 1980
6) SHOJI, J.; H. HINOO, R. SAKAZAKI, N. TSUJI, K. NAGASHIMA, K. MATSUMOTO, Y. TAKAHASHI, S. KOZUKI, T. HATTORI, E. KONDO & K. TANAKA: Asparenomycins A, B and C, new carbapenem antibiotics. II. Isolation and chemical characterisation. J. Antibiotics 35: 15~23, 1982
7) OKABE, M.; S. AZUMA, I. KOJIMA, K. KOUNO, R. OKAMOTO, Y. FUKAGAWA & T. ISHIKURA: Studies on the OA-6129 group of antibiotics, new carbapenem compounds. I. Taxonomy, isolation and physical properties. J. Antibiotics 35: 1255~1263, 1982
8) KROPP, H.; J.G. SUNDELOF, R. HAJDU & F.M. KAHAN: Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-1. Antimicrob. Agents Chemother. 22: 62~70, 1982
9) GRAHAM, D. W.; F. M. KAHAN & E. F. ROGERS (Merck & Co., Inc.): Novel *Z*-2-acylamino-3-monosubstituted propenic acids and their esters and salts, and processes for preparing them. European Patent 10,573, May 1980
10) KAHAN, F. M. & H. KROPP (Merck & Co., Inc.): Antibacterial compositions comprising a β-lactam type compound and a 3-substituted propenoate. European Patent 28,778, May 1981
11) KROPP, H.; J. G. SUNDELOF, D. L. BOHN & F. M. KAHAN: Improved urinary-tract bioavailability of MK 0787 when co-administered with inhibitors of renal dihydropeptidase-I. Abstract 270, 20th Intersci. Conf. Antimicrob. Agents and Chemother., New Orleans, 1980
12) NORRBY, S. R.; K. ALESTIG, B. BJÖRNEGÅRD, L. Å. BURMAN, F. FERBER, J. L. HUBER, K. H. JONES, F. M. KAHAN, J. S. KAHAN, H. KROPP, M. A. P. MEISINGER & J. G. SUNDELOF: Urinary recovery of *N*-formimidoylthienamycin (MK 0787) as affected by co-administration of *N*-formimidoylthienamycin dehydropeptidase inhibitors. Antimicrob. Agents Chemother. 23: 300~307, 1983
13) FERRES, H.: Pro-drugs of β-lactam antibiotics. Drugs of Today 19: 499~538, 1983
14) BALTZER, B.; E. BINDERUP, W. VON DAEHNE, W. O. GODTFREDSEN, K. HANSEN, B. NIELSEN, H. SØRENSEN & S. VANGEDAL: Mutual pro-drugs of β-lactam antibiotics and β-lactamase inhibitors. J. Antibiotics 33: 1183~1192, 1980
15) FUCHS, K. & E. KATSCHER: Symmetrical dichlorodimethyl sulphate, chloromethyl chlorosulphonate and similar derivatives of formaldehyde. Chem. Ber. 60B: 2288, 1927