SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME 6α-FORMAMIDO PENICILLINS MODIFIED IN THE 2β-METHYL GROUP

Sir:

Recent publications[1,2] have highlighted the discovery of potent β-lactamase inhibitors by modification of the 2β-methyl group in sulbactam. Similar modification of penicillins, with intact 6β-acylamino substituents[3,4], has been less successful in providing potent antibacterial agents. Earlier work in these laboratories has demonstrated how the introduction of a 6α-formamido substituent[5] and a catechol[6] into penicillins gives rise to potent antibacterial agents with β-lactamase stability. In this paper we describe the incorporation of a number of substituents including a catechol, into the 2β-methyl group of 6α-formamido penicillins. The effect of the 2-substituent on antibacterial activity will be highlighted.

The key intermediate in the preparation of 2-modified 6α-formamido penicillins is the azetidinone disulfide **4**. This was readily available from the protected penicillin[5] **1** by modification of a literature procedure[7]. The presence of a 6α-formamido substituent results in a mixture of α- and β-sulfoxides **2** and **3**[8] upon oxidation of **1** with *m*-chloroperoxybenzoic acid. Thermolysis of either of the pure isomers, or a mixture, in the presence of benzothiazole-2-thiol resulted in trapping of the intermediate sulfenic acid providing the disulfide **4**. The conversion of **4** into 2β-substituted penicillins was achieved by two general methods. In the first, disulfide **4** was treated with a solution of bromine in carbon tetrachloride containing acetamide. The 2β-bromomethyl penam **5a** was obtained in 63% yield. Introduction of a 1-methyltetrazol-5-ylthio substituent was achieved by reaction of **5a** with sodium 1-methyltetrazol-5-yl thiolate in acetone-pH 6.9 phosphate buffer and provided **5b**. Treatment of **5a** with silver tetrafluoroborate in methanol resulted in replacement of bromo by methoxy to give **5c**. The second general method involved reaction of disulfide **4** with pre-formed complexes of iodine and the silver salt of a carboxylic acid[9]. Thus benzoic acid provided the 2β-benzoyloxymethyl derivative **5d**. Incorporation of a catechol was accomplished by reaction of **4** with iodine and silver 3,4-diacetoxybenzoate to give **5e**. Removal of the trichloroethoxycarbonyl protecting group from compounds **5b**~**5e** using zinc in pH 4 phosphate buffer provided the modified penicillin nuclei **6b**~**6e**. Coupling of **6b**~**6e** to *R*,2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetyl chloride to give **7b**~**7e** was significantly slower than for the parent nucleus **6g**. This may be a direct result of steric hindrance by the 2β-substituent. Subsequently the preparation of a larger quantity of **5e** resulted in an inseparable 4:1 mixture of **5e** and a compound thought to be the isomeric cepham. After deprotection and acylation of the mixture compounds **7e** and the cepham **9** were separated by column chromatography. Hydrogenolysis of the benzyl ester in compounds **7b**~**7e** and **9** was followed by neutralisation providing the sodium salts **8b**~**8e** and **10**. The acetyl protecting groups of compound **8e** were removed by exposure to purified citrus acetylesterase to give the catechol containing derivative **8f**.

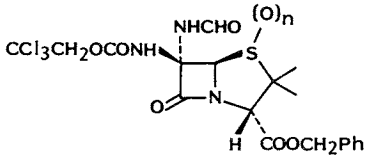
The antibacterial activities of the 2-substituted penicillin derivatives, compared with that of the

Table 1. MICs[a] of 2β-substituted 6α-formamido penicillins.

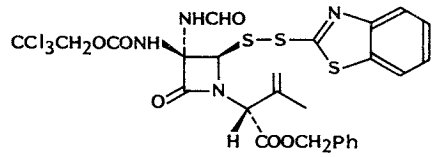
Organism	MIC (μg/ml)						
	8b	**8c**	**8d**	**8e**	**8f**	**8g**	**10**
Escherichia coli DCO	16	>64	>64	0.12	0.12	4.0	>64
E. coli DCO RTEM	16	>64	>64	0.5	0.25	4.0	>64
Enterobacter cloacae N1	32	>64	>64	4.0	4.0	2.0	>64
E. cloacae P99	32	>64	>64	4.0	2.0	8.0	>64
Klebsiella pneumoniae T767	32	>64	>64	0.25	0.12	4.0	>64
Pseudomonas aeruginosa NCTC 10662	128	>64	>64	0.5	0.5	16	>64
P. aeruginosa Dalgleish	128	>64	>64	0.5	0.5	16	>64
P. aeruginosa Badia	>128	>64	>64	2.0	1.0	32	>64
Staphylococcus aureus Oxford	>128	>64	>64	>64	>128	>128	>64
Streptococcus pyogenes CN10	1.0	>64	64	8.0	4.0	2.0	>64

[a] MIC determined in Iso-Sensitest Agar (Oxoid) inoculated with 10^4 cfu/ml.

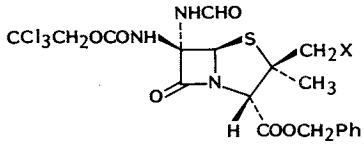
parent compound **8g** are shown in Table 1. It will be seen that incorporation of methoxy, benzoyloxy or 1-methyltetrazol-5-ylthio groups into the β-methyl group results in compounds that are either inactive or show significantly reduced activity. In contrast to the poor activity of the benzoyloxy derivative **8d**, introduction of a catechol function as the 3,4-dihydroxybenzoyloxy moiety provides a compound **8f**, with potent activity. The latter is substantially more active against strains of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* than the unsubstituted compound **8g**.



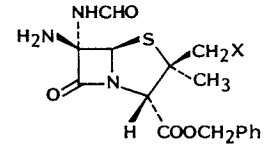
1 n=0
2 n=1, α-isomer
3 n=1, β-isomer



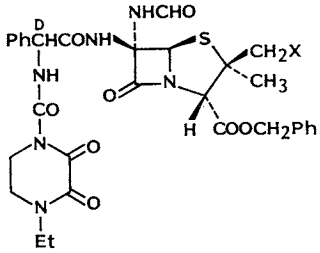
4



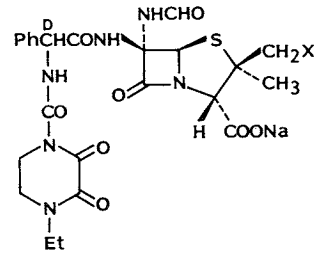
5a~5e



6b~6e, 6g

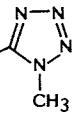


7b~7e

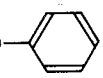


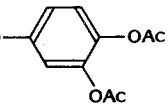
8b~8g

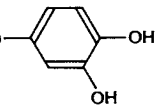
a X=Br

b X= 

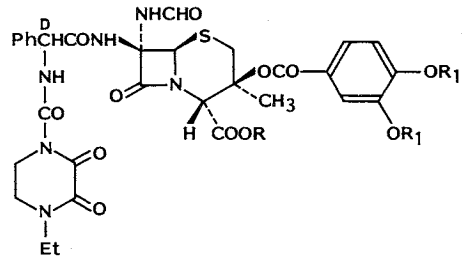
c X=OMe

d X= 

e X= 

f X= 

g X=H



9 R=CH_2Ph R_1=Ac
10 R=Na R_1=Ac

The increase in potency observed with the catecholic compound is probably the result of uptake of **8f** *via* the bacterial iron transport mechanism[10].

The difference in intrinsic activities of the penam **8e** and cepham **10** systems is also shown in Table 1. While the former has potent activity the latter is essentially inactive despite the presence of a catechol function.

Acknowledgements

We are most grateful to Mrs. S. J. KNOTT and Miss J. LEMMER for the antibacterial susceptibility testing.

DAVID F. CORBETT
COLIN H. FRYDRYCH*
ROBERT SOUTHGATE
MICHAEL J. BASKER

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

(Received February 5, 1990)

References

1) HALL, T. W.; S. N. MAITI, R. G. MICETICH, P. SPEVAK, S. YAMABE, N. ISHIDA, M. KAJITANI, M. TANAKA & T. YAMASAKI: YTR-830 and related active β-lactamase inhibitors. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Eds.*, A. G. BROWN & S. M. ROBERTS, pp. 242~254, Third International Symposium, Cambridge, 1985

2) GOTTSTEIN, W. J.; L. B. CRAST, Jr., R. G. GRAHAM, U. J. HAYNES & D. N. MCGREGOR: Synthesis and β-lactamase inhibitory properties of 2β-(chloromethyl)-2α-methylpenam-3α-carboxylic acid, 1,1-dioxide. J. Med. Chem. 24: 1531~1534, 1981

3) KAMIYA, T.; T. TERAJI, M. HASHIMOTO, O. NAGAGUTI & T. OKU (Fujisawa): Penam and cepham derivatives and preparation thereof. U. S. P 3,954, 732, May 4, 1976

4) HASHIMOTO, M. & T. KAMIYA: Recent chemical modification of β-lactam antibiotics. Jpn. J. Antibiotics 30 (Suppl.): S218~S229, 1977

5) SMALE, T. C.; A. W. GUEST, F. P. HARRINGTON, P. H. MILNER, R. J. PONSFORD & A. V. STACHULSKI: 6α (7α)-Formamido penicillins and cephalosporins. J. Chem. Soc. Chem. Commun. 1984: 1335~1336, 1984

6) BASKER, M. J.; R. A. EDMONDSON, S. J. KNOTT, R. J. PONSFORD, B. SLOCOMBE & S. J. WHITE: In vitro antibacterial properties of BRL 36650, a novel 6α-substituted penicillin. Antimicrob. Agents Chemother. 26: 734~740, 1984

7) KAMIYA, T.; T. TERAJI, Y. SAITO, M. HASHIMOTO, O. NAKAGUCHI & T. OKU: Studies on β-lactam antibiotics. I. A novel conversion of penicillins into cephalosporins. Tetrahedron Lett. 1973: 3001~3004, 1973

8) BRANCH, C. L.; M. J. PEARSON & T. C. SMALE: Direct incorporation of a 6α(7α)-formamido group into penicillins and cephalosporin sulphides and sulphoxides. J. Chem. Soc. Perkin Trans. I 1988: 2865~2873, 1988

9) GOTTSTEIN, W. J.; U. J. HAYNES & D. N. MCGREGOR: Synthesis and β-lactamase inhibitory properties of 2β-[(acyloxy)methyl]-2α-methylpenam-3α-carboxylic acid, 1,1-dioxides. J. Med. Chem. 28: 518~522, 1985

10) CURTIS, N. A. C.; R. L. EISENSTADT, S. J. EAST, R. J. CORNFORD, L. A. WALKER & A. J. WHITE: Iron-regulated outer membrane proteins of *Escherichia coli* K-12 and mechanism of action of catechol-substituted cephalosporins. Antimicrob. Agents Chemother. 32: 1879~1886, 1988