IN VITRO BIOLOGICAL ACTIVITIES OF 6-ISOSTERIC PENICILLINS AND 7-ISOSTERIC CEPHALOSPORINS

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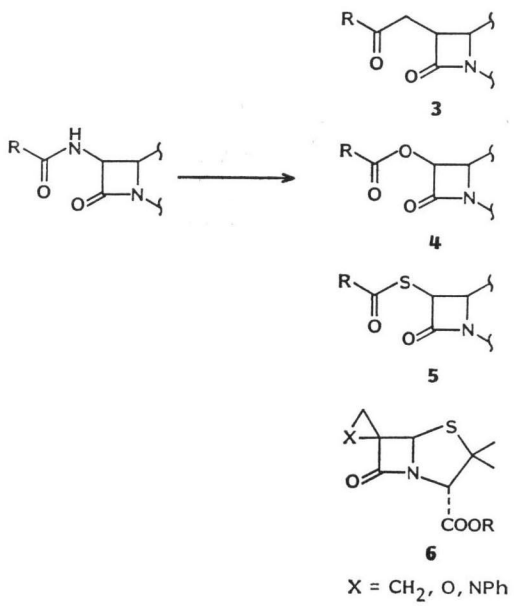
Antibiotic and penicillinase inhibitor activities of various penicillin and cephalosporin analogs are reported. The compounds include C-6 penicillin and C-7 cephalosporin carbon, oxygen and sulfur analogs obtained by replacing the NH of the amide side chains with CH_2, O and S, respectively. In almost all cases, analogs were considerably less active than the standard compounds (benzylpenicillin and cephalothin). However, some of the analogs act as penicillinase inhibitors.

The search for structure-activity relationships in β-lactam antibiotics has proceeded through programs for isolation of new compounds[1~3] as well as chemical modification of existing antibiotics[4,5]. We wish to contribute to this effort by reporting on the *in vitro* biological activities of various penicillin (**1**) and cephalosporin (**2**) analogs synthesized in our laboratory.

Our efforts toward structural modifications have targeted mainly the C-6(7) position of penicillin (cephalosporin). We have arrived at new compounds by replacing the amide side chains with carbon[6~8], oxygen[9], and sulfur[10,11] side chains (see Scheme 1) and have also been successful in synthesizing several C-6 spiro peni-

Scheme 1.



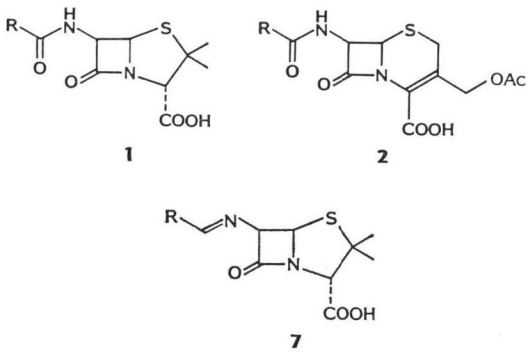
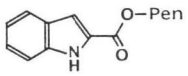
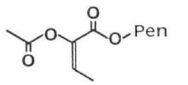
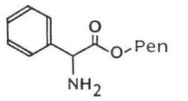
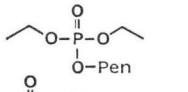
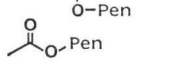
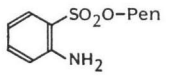
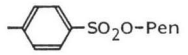
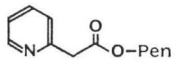
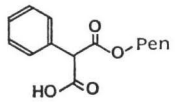
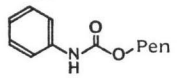
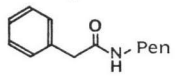
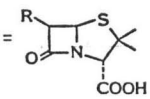


Table 1. MIC (μg/ml) of C-6 oxygen analogs of penicillin which show only Gram-positive activity.

Structure*	BS**	ML	SA-100	SA	SE	SP-58***	SP-59	SF	SP
	3.1	6.3	NT****	6.3	13	NT	NT	NT	6.3
$CH_3(CH_2)_7COO$–Pen	3.1	3.1	NT	3.1	NT	>200	NT	NT	3.1
	4.7	NT	NT	3.1	6.3	NT	NT	NT	150
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COO$–Pen	6.3	13	NT	25	NT	>200	NT	NT	13
	<0.4	NT	NT	NT	NT	100	<0.4	>200	NT
	0.8	>200	1.6	NT	NT	NT	NT	>200	NT
	50	NT	6.3	NT	NT	>200	NT	>200	NT
	25	NT	38	NT	NT	NT	NT	>200	NT
	NT	150	100	NT	NT	>200	NT	>200	NT
	20	NT	>200	NT	NT	>200	NT	>200	NT
	NT	NT	>200	NT	NT	>200	NT	>200	NT
	50	NT	>200	NT	NT	>200	NT	>200	NT
 (Benzylpenicillin control)	<0.4	<0.4	<0.4	<0.4	<0.4	3.1	<0.4	3.1	<0.4

* R–Pen = 

** BS; *Bacillus subtilis* ATCC 6051, ML; *Micrococcus luteus* ATCC 8341, SA-100; *Staphylococcus aureus* A-100, SA: *S. aureus* ATCC 25923, SE; *S. epidermidis* ATCC 14990, SP-58; *S. pyogenes* MIT B-58, SP-59; *S. pyogenes* MIT B-59; SF; *Streptococcus faecalis* MIT B-57, SP; *S. pyogenes* ATCC 10389.

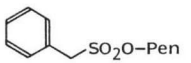
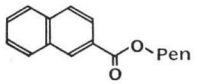
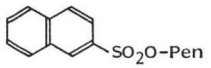
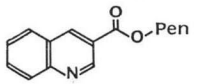
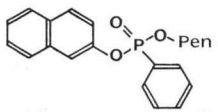
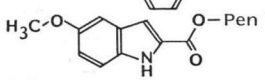
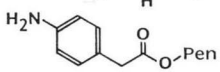
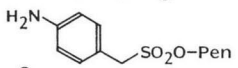
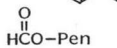
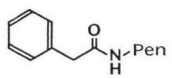
*** Penicillin-resistant strain.

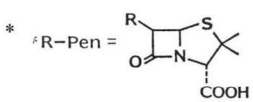
**** NT=not tested.

cillins (**6**)[12,13]. The biological properties of these non-amide penicillins and cephalosporins are the focus of the present report.

Among the non-amide penicillins, β-amidino compounds (for example, mecillinam (**7**)) are known to possess excellent antibiotic activity[14]. Following the isolation of 7-methoxycephalosporins[15], which are stable toward β-lactamases, a large number of C-6(7)-substituted penicillins (cephalosporins) have been synthesized. Cephalosporins[5] with groups such as OCH_3, SCH_3, CH_2OH, OCH_2CH_3, CHO,

Table 2. MIC (μg/ml) of C-6 oxygen analogs of penicillin which possess some Gram-negative activity.

Structure*	BS**	ML	SA-100	SA	SE	SP-58***	SF	SP	EC	KP	PM	PV	SEN	SS
	0.8	0.8	0.8	NT****	NT	150	200	NT	>200	25	200	NT	50	>200
	<6.3	<6.3	<6.3	0.8	1.6	25	25	1.6	200	NT	25	>200	>200	>200
H_3CSO_2O–Pen	50	NT	6.3	NT	NT	>200	>200	NT	200	200	>200	200	NT	NT
	<3.1	NT	<3.1	NT	NT	100	100	NT	>200	>200	100	>200	>200	>200
	<3.1	NT	<3.1	NT	NT	100	100	NT	>200	>200	100	>200	>200	>200
	<3.1	NT	<3.1	NT	NT	100	100	NT	>200	NT	100	>200	>200	>200
	<3.1	NT	13	NT	NT	50	50	NT	>200	>200	50	>200	>200	>200
	0.8	1.6	3.1	NT	NT	NT	200	NT	200	>200	200	>200	100	200
	25	13	100	NT	NT	NT	>200	NT	150	NT	>200	>200	200	200
	1.6	NT	200	NT	NT	>200	200	NT	100	200	>200	>200	NT	NT
 (Benzylpenicillin control)	<0.4	<0.4	<0.4	<0.4	<0.4	3.1	3.1	<0.4	25	100	6.3	200	6.3	100

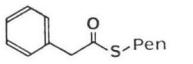
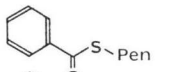
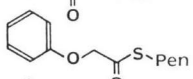
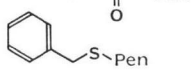
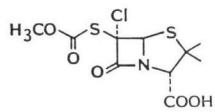
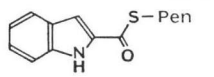
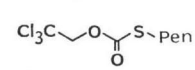
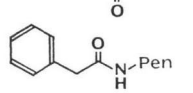
* 

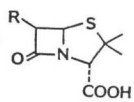
** EC; *Escherichia coli* ATCC 8739, KP; *Klebsiella pneumoniae* MIT B-49, PM: *Proteus mirabilis* MIT B-52, PV; *P. vulgaris* ATCC 6380, SEN; *Salmonella enteritidis* A9531, SS; *Shigella sonnei* ATCC 1106. See also footnote of Table 1.

*** Penicillin-resistant strain.

**** NT=not tested.

Table 3. MIC (μg/ml) of C-6 sulfur analogs of penicillin.

Structure*	*B. subtilis* ATCC 6051	*M. luteus* ATCC 9341	*S. sonnei* ATCC 11060	Other strains
	<0.4	NT**	NT	Inactive (>200) vs. *S. aureus* A100, *S. pyogenes* MIT B-58, *S. faecalis* MIT B-57, *E. coli* ATCC 8739, *S. enteritidis* A9531, *P. mirabilis* MIT B-52, *P. vulgaris* ATCC 6380, *K. pneumoniae* MIT B-49
	<0.4	NT	NT	
	50	NT	NT	
	100	100	100	
	200	100	100	
	>200	100	>200	Inactive (>200) vs. *S. aureus* A100, *E. coli* ATCC 8739, *S. enteritidis* A9531, *P. mirabilis* MIT B-52, *P. vulgaris* ATCC 6380, *K. pneumoniae* MIT B-49
	100	200	>200	
 (Benzylpenicillin control)	<0.4	<0.4	100	

* R–Pen = 

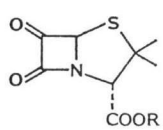
** NT=not tested.

$COCH_3$, NHCOOEt, CH_3 and CH(ph)OH at C-7 and penicillins with Cl, Br, OH, CH_3, OCH_3 and $CH_3CH(OH)$ at C-6 are among them[5]. The effects of these modifications at C-6(7) and other positions of penicillin (cephalosporin) on biological activities have been detailed in recent reviews[4,5].

Materials and Methods

Materials

All of the compounds reported here have been synthesized[6~13] in our laboratory. Highly reactive 6 (7)-ketocompounds (**8** and **9**) have been employed in the synthesis of the carbon and oxygen analogs. Synthesis of **8** and **9** and conversions to carbon analogs using Wittig-type reactions and to oxygen analogs *via* reduction followed by acylation have been detailed previously[6~9]. The 6-spiro penicillins and sulfur analogs were synthesized[10~13] from 6-diazo intermediates (**10**) using electrophilic reagents. Benzylpenicillin was provided by Merck and Company, Inc. Cephalothin was manufactured by Eli Lilly & Co. Ampicillin was a gift from Ayerst Laboratories. Organisms used for testing were either obtained from Ayerst Laboratories as gifts or purchased from Roche Diagnostics.



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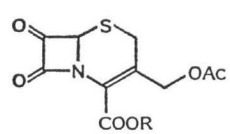

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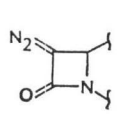
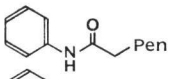
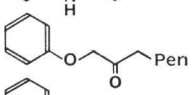
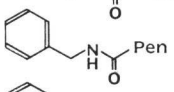
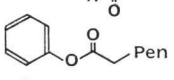
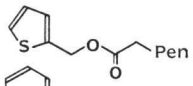
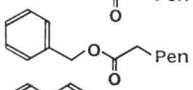
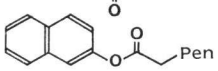
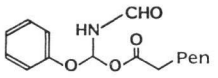
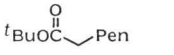
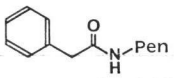
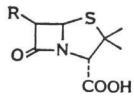


10

Table 4. MICs of C-6 carbon analogs of penicillins (μg/ml).

Structure*	BS**	ML	SA-100	SP-58***	SF	EC	KP	PM	PV
	6.3	NT****	13	200	200	>200	100	200	200
	0.8	100	25	NT	>200	>200	>200	>200	>200
	3.1	13	100	200	NT	>200	>200	>200	>200
	50	50	25	NT	NT	>200	>200	>200	>200
	13	NT	6.3	>200	>200	>200	>200	>200	>200
	21	NT	5.7	>200	>200	NT	NT	>200	>200
	13	NT	25	>200	>200	>200	>200	>200	>200
	6.3	NT	13	200	>200	>200	>200	200	>200
	NT	6.3	50	>200	>200	>200	NT	>200	>200
 (Benzylpenicillin control)	<0.4	<0.4	<0.4	3.1	3.1	25	100	6.3	200

* R–Pen = 

** See footnotes of Tables 1 and 2.

*** Penicillin-resistant strain.

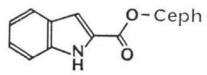
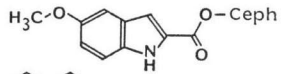
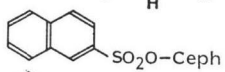
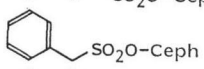
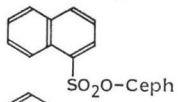
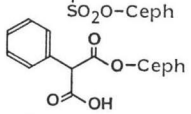
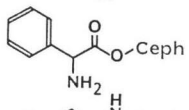
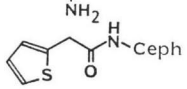
**** NT=not tested.

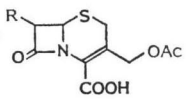
Biological Tests

Minimum Inhibitory Concentration (MIC): Trypticase soy agar plates were prepared containing concentrations of sample or standard ranging from 0 to 200 μg/ml agar. All solutions were prepared and diluted in either dimethyl sulfoxide or water. Each plate was inoculated with all organisms using a Steers replicator. The organisms used were as follows: Gram-positive bacteria: *Staphylococcus aureus* strains A100 and ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus pyogenes* strains Pen R (MIT B-58) and B-59, *Streptococcus pyogenes* ATCC 10389, *Bacillus subtilis* ATCC 6051, *Micrococcus luteus* ATCC 9341, *Streptococcus faecalis* MIT-B57. Gram-negative bacteria: *Escherichia coli* strains ATCC 25922 and ATCC 8739, *Salmonella enteritidis* A9531, *Proteus mirabilis* MIT B-52, *Proteus vulgaris* ATCC 6380, *Klebsiella pneumoniae* strains ATCC 27736 and MIT-B49, *Shigella sonnei* ATCC 11060. The plates were incubated for 18 hours at 37°C. MIC was the lowest level which inhibited growth.

Inhibition of Penicillinase: Trypticase soy agar was prepared containing either no antibiotic, ampicillin (5 μg/ml), or benzylpenicillin (10 μg/ml). All of the above were seeded with *Klebsiella aerogenes* ATCC 15380, a penicillinase producer. Sample compounds were prepared at 5 mg/ml. Two known active standards (BRL 1437[16] and clavulanic acid) were prepared at 1 mg/ml and 10 μg/ml, respectively. Filter paper disks (6.35 mm) were dipped into the test solutions and placed on the agar plates. Each plate also had disks containing clavulanic acid and BRL 1437. Plates were incubated for

Table 5. MICs (μg/ml) of C-7 oxygen analogs of cephalosporin.

Structure*	BS**	ML	SA-100	SA	SE	SP-58***	SF	SP
	<1.6	6.3	NT****	3.1	3.1	NT	NT	3.1
	<1.6	13	NT	6.3	13	NT	NT	6.3
	25	25	NT	3.1	25	NT	NT	>200
	13	NT	13	NT	NT	>200	>200	NT
	25	100	NT	100	>200	NT	NT	>200
	6.3	NT	100	NT	NT	>200	>200	NT
	50	NT	>200	NT	NT	>200	>200	NT
	<0.4	<0.4	<0.4	<0.4	<0.4	50	50	<0.4
(Cephalothin control)								

* R-Ceph = 

** See footnotes of Tables 1 and 2.

*** Penicillin-resistant strain.

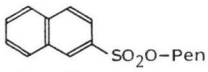
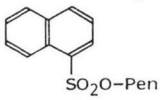
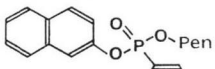
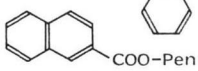
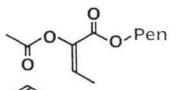
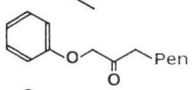
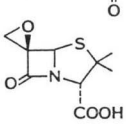
**** NT=not tested.

18 hours at 37°C. A compound with penicillinase-inhibitory activity produces a larger zone of inhibition on one or both of the antibiotic-containing plates than on the unsupplemented plate. *K. aerogenes*, BRL 1437 and clavulanic acid were gifts of Beecham Research Laboratories.

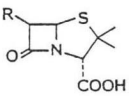
Results and Discussion

MIC values of the active compounds along with those of the standards are listed in Tables 1~5. As a general rule, the antibacterial activity of the compounds decreased in the order: oxygen analog> carbon analog>sulfur analog. Some of the analogs of penicillins possessed weak activity against Gram-negative bacteria (Table 2). Cephalosporin oxygen analogs (Table 5), penicillin carbon analogs (Table 4) and certain of the penicillin oxygen analogs listed in Table 1 were only active against Gram-positive organisms. Although most of the analogs tested show some activity, they are considerably less potent than the parent amide compounds. However, some showed interesting activity as inhibitors of penicillinase (Table 6), although none of these analogs was more active than clavulanic acid.

Table 6. C-6 analogs of penicillin which inhibit the penicillinase of *K. aerogenes**.

Structure**	Zone diameter (mm)		
	Compound alone	Compound + ampicillin***	Compound + benzylpenicillin***
	<7	16.7	20.0
	<7	12.4	15.9
	<7	11.0	12.6
	<7	9.5	trace
	7.2	9.0	9.2
	<7	11.5	12.7
	<7	9.4	9.8

* Controls were carried out with known penicillinase inhibitors. BRL 1437 (disk saturated with 1 mg/ml solution) produced <7 mm zone alone, 12.2 mm zone with ampicillin and 13.1 mm zone with benzylpenicillin. Clavulanic acid (disk saturated with 10 μg/ml solution) produced <7 mm zone alone, 19.4 mm zone with ampicillin and 18.8 mm zone with benzylpenicillin.

** R–Pen = 

*** Ampicillin and benzylpenicillin were present in the agar at 5 μg/ml and 10 μg/ml respectively.

References

1) Brown, A. G.; D. Butterworth, M. Cole, G. Hanscomb, J. D. Hood & C. Reading: Naturally-occurring β-lactamase inhibitors with antibacterial activity. J. Antibiotics 29: 668~669, 1976
2) Aoki, H.; H. Sakai, M. Kohsaka, T. Konomi, J. Hosoda, Y. Kubochi, E. Iguchi & H. Imanaka: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976
3) Kahan, J. S.; F. M. Kahan, E. O. Stapley, R. T. Goegelman & S. Hernandez: Antibiotics. U.S. Patent 3,950,357, Apr. 13, 1976
4) Gorman, M. & C. W. Ryan: Structure-activity relationships of β-lactam antibiotics. *In* Cephalosporins and Penicillins. *Ed.*, Flynn, E. H., pp. 532~582, Academic Press, New York, 1972
5) Cama, L. D. & B. G. Christensen: Structure-activity relationships of non-classical β-lactam antibiotics. *In* Annual Reports in Medicinal Chemistry. *Ed.*, Whitfield, G., pp. 149~158, Academic Press, New York, 1978
6) Sheehan, J. C.: Chemical modifications of the β-lactam ring of penicillins and cephalosporins. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.*, Elks, J., pp. 20~24, The Chemical Society, London, 1977
7) Sheehan, J. C. & Y. S. Lo: Benzyl 6-oxopenicillanate and derivatives. II. J. Org. Chem. 38: 3227~3228, 1973

8) SHEEHAN, J. C.; A. BUKU, E. CHACKO, T. J. COMMONS, Y. S. LO, D. R. PONZI & W. SCHWARZEL: Derivatives of 6β-methylpenicillanic acid. J. Org. Chem. 42: 4045~4048, 1977
9) LO, Y. S. & J. C. SHEEHAN: Synthesis of benzyl 6-oxopencillanate and derivatives. I. J. Am. Chem. Soc. 94: 8253, 1972
10) SHEEHAN, J. C.; T. J. COMMONS & Y. S. LO: Photochemical conversion of β,β,β-trichloroethyl 6-diazopenicillanate into 6β-thiolpenicillin derivatives. J. Org. Chem. 42: 2224~2229, 1977
11) SHEEHAN, J. C. & T. J. COMMONS: Reaction of 6(7)-diazopenicillanates and diazocephalosporanates with sulfenyl chlorides. Preparation of 6(7)α-methoxy-substituted thiol penicillanates and thiol cephalosporanates. J. Org. Chem. 43: 2203~2208, 1978
12) SHEEHAN, J. C.; E. CHACKO, Y. S. LO, D. R. PONZI & E. SATO: Some new spiro penicillins. J. Org. Chem. 43: 4856~4859, 1978
13) SHEEHAN, J. C.; K. NAKAJIMA & E. CHACKO: Reactions of β,β,β-trichloroethyl 6-diazopenicillanate with aldehydes and Schiff bases. Heterocycles 13: 227~234, 1979
14) LUND, F. J.: 6β-Amidinopencillanic acids-synthesis and antibacterial properties. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.*, ELKS, J., pp. 25~45, The Chemical Society, London, 1977
15) NAGARAJAN, R.; L. D. BOECK, M. GORMAN, R. I. HAMILL, C. E. HIGGINS, M. M. HOEHN, W. M. STARK & J. G. WHITNEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 93: 2308~2312, 1971
16) COLE, M.; S. ELSON & P. D. FULLBROOK: Inhibition of the β-lactamases of *Escherichia coli* and *Klebsiella aerogenes* by semi-synthetic penicillins. Biochem. J. 127: 295~308, 1972