IN VITRO ACTIVITY OF CEPHALOSPORINS AGAINST *MYCOBACTERIUM TUBERCULOSIS* H37Rv: STRUCTURE-ACTIVITY RELATIONSHIPS

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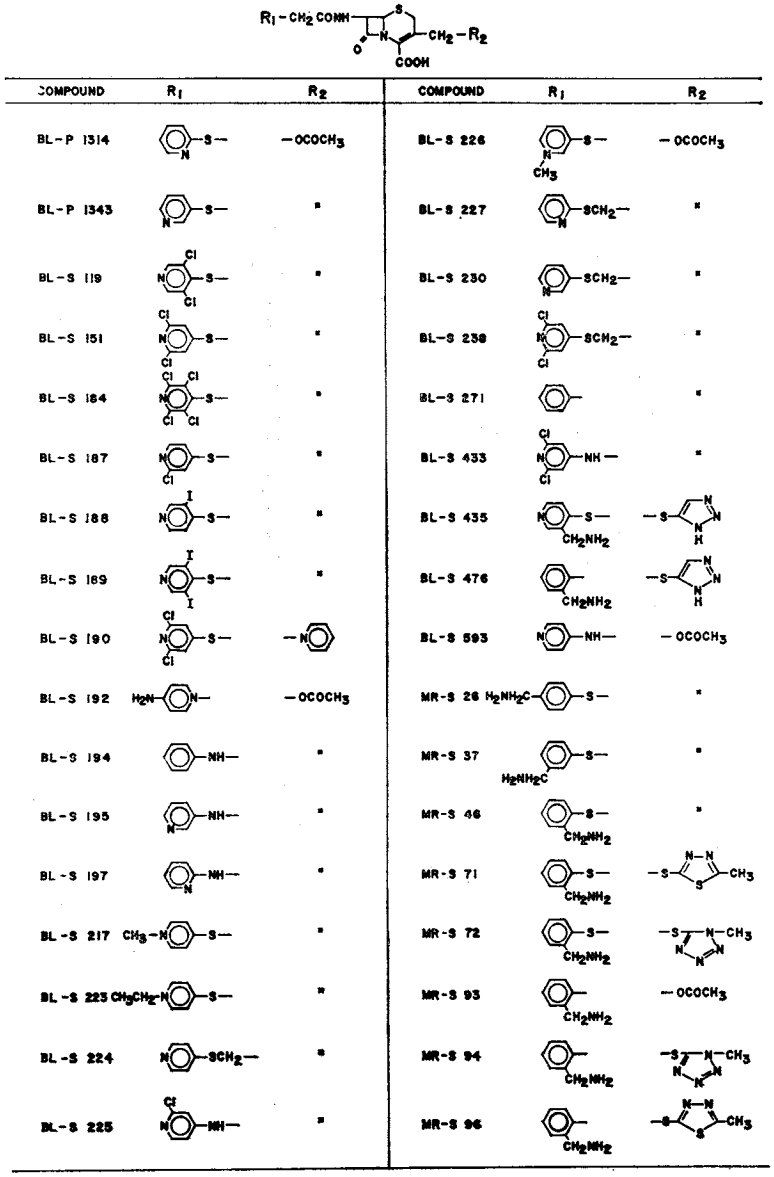
Over 600 derivatives of cephalosporin C were screened for activity against *Mycobacterium tuberculosis* H37Rv by a 2-fold broth dilution method using DUBOS liquid medium. Among the most active derivatives were those in which a pyridyl or an aminomethylphenyl moiety was present in the 7-side chain. Structure-activity relationships have been determined for a number of such compounds of which 34 were considered in this report. In the pyridyl series, consideration was given to the effect on activity of the position of the nitrogen in the pyridine ring, N-alkylation and halogenation of this ring system, as well as the influence of a sulfur or an amino bridge in the 7-side chain. The most active derivative in the pyridyl series was a chlorinated analogue of cephapirin, 7-[(2,6-dichloropyrid-4-yl) thioacetamido] cephalosporanic acid, whose minimal inhibitory concentration of 1.4 μg/ml was 10 times less than that of cephapirin and only twice that of dihydrostreptomycin. Several other derivatives in the pyridyl series whose activity was comparable to that of the chlorinated analogue of cephapirin had pyrid-4-ylaminoacetamido or 4-aminopyrid-1-ylacetamido 7-side chains. Relationships considered in the aminomethylphenyl series were the effect on activity of the position of the aminomethyl group on the phenyl ring, the presence or absence of a sulfur bridge in the 7-side chain, as well as the influence of substitutions of heterocyclic-thiomethyl groups for the acetoxymethyl moiety at the 3-position. All derivatives in this series having activity comparable to that of the chlorinated analogue of cephapirin contained an *o*-aminomethylphenylacetamido side chain at the 7-position.

A search of the literature shows that *in vitro* testing of semisynthetic cephalosporins and penicillins for antimycobacterial activity has been essentially confined to those compounds which have been approved for clinical use[1,2,3,4,5]. As a consequence of the poor antimycobacterial activity displayed by these compounds, *Mycobacterium tuberculosis* is usually not one of the microorganisms routinely used in screening new derivatives of β-lactam antibiotics for antibacterial activity. It seems likely that the poor activity of these commercially available antibiotics against members of the genus *Mycobacterium* may be due to the β-lactamases produced by the various species[6,7,8,9]. However, the availability of 7-aminocephalosporanic acid and 6-aminopenicillanic acid has permitted the preparation of numerous new cephalosporins and penicillins in the laboratory that possess increasingly greater antibacterial activity, broad or selective, as well as a higher degree of resistance to β-lactamases. Recognizing the continuing need for new first-line as well as second-line antitubercular drugs, we have for the past several years routinely screened new antibiotics for activity *in vitro* against *M. tuberculosis* H37Rv. This presentation reports on the antimycobacterial activity and structural relationships of a group of semisynthetic cephalosporins. The structures of the 34 compounds to be discussed, excluding those of cephalosporins which are generically identified, are shown in Fig 1.

Materials and Methods

The cephalosporin C derivatives prefixed with the letters "BL-P" or "BL-S" were synthesized

Fig. 1. Structures of semisynthetic cephalosporins identified by code.



by L. B. CRAST, R. G. GRAHAM, J. M. ESSERY and U. CORBIN at Bristol Laboratories, while those coded "MR-S" were prepared at R. & L. Molecular Research Ltd. of Canada. All of the coded cephalosporins were at least 80% pure, and soluble in water, aqueous bicarbonate or dimethylsulfoxide. The generically identified cephalosporins used are products of Bristol Laboratories (cephapirin), Ciba Pharmaceutical Co. (cephacetrile), Fujisawa Pharmaceutical Co. of Osaka, Japan (cefazolin), Eli Lilly & Co. (cephalothin, cephaloridine, cephaloglycin, cephalexin, cephanone and cefamandole), Merck & Co. (cefoxitin) and E.R. Squibb & Sons (cephradine). Dihydrostreptomycin (Eli Lilly & Co.) was used to monitor the sensitivity of the *Mycobacterium tuberculosis* H37Rv culture.

The susceptibility of *M. tuberculosis* H37Rv to the antibiotics was determined by a 2-fold broth dilution method using DUBOS Liquid Medium (Difco). The culture was grown for 7~10 days at 37°C in this medium and then diluted to 2% (v/v) for a working inoculum which was then used at

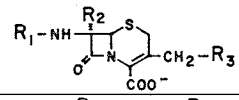
the rate of 3 ml per one ml of medicated medium in 15×150 mm test tubes. The minimal inhibitory concentration (MIC) of drug was determined after incubation at 37°C for 5 days.

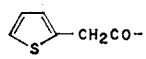
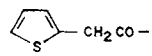
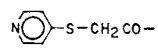
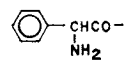
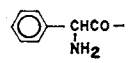
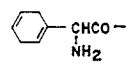
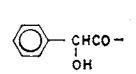
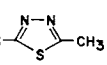
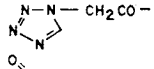
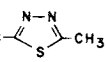
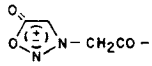
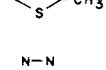
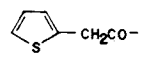
Results and Discussion

Results comparing the antimycobacterial activity of a group of cephalosporins that are either commercially available or are promising candidates for clinical use are shown in Table 1. Dihydrostreptomycin and 7-aminocephalosporanic acid (7-ACA) were included in the study as control compounds.

Of the cephalosporins studied, only cephapirin, cephaloridine, cephanone and cefazolin were more active than 7-ACA. Cephapirin and cephaloridine, each containing a pyridine ring, were the most active, and were comparable in activity. They were about 4-times more active than 7-ACA, and about twice as active as cefazolin and cephanone, two derivatives having heterocyclic groups in both the 3- and 7-side chains. Cephapirin and cephaloridine, when compared to dihydrostreptomycin, were only about 1/25 as active.

Table 1. Activity of various cephalosporins against *M. tuberculosis* H37Rv in DUBOS medium.



CEPHALOSPORIN	R_1	R_2	R_3	MIC*(μg/ml)
7-AMINOCEPHALOSPORANIC ACID	H-	-H	$-OCOCH_3$	59
CEPHALOTHIN		-H	$-OCOCH_3$	63
CEPHALORIDINE		-H		16
CEPHAPIRIN		-H	$-OCOCH_3$	13
CEPHACETRILE	$N\equiv C-CH_2CO-$	-H	$-OCOCH_3$	57
CEPHALOGLYCIN		-H	$-OCOCH_3$	200
CEPHALEXIN		-H	-H	100
CEPHRADINE		-H	-H	100
CEFAMANDOLE		-H		52
CEFAZOLIN		-H		33
CEPHANONE		-H		25
CEFOXITIN		$-OCH_3$	$-OCONH_2$	100
DIHYDROSTREPTOMYCIN				0.6

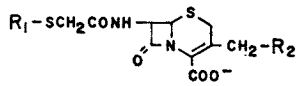
* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 6 DETERMINATIONS).

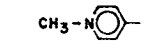
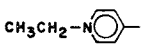
Cephalothin, cephacetrile and cefamandole were about as active as 7-ACA, while cefoxitin, a 7-methoxycephalosporin, and the α-aminocephalosporins, cephaloglycin, cephlexin, and cephradine, were all substantially less active than 7-ACA.

A comparison of the antimycobacterial activity of a number of analogues of cephapirin, sodium 7-(pyrid-4-ylthioacetamido) cephalosporanate, is shown in Table 2. It can be seen that the position of the nitrogen in the pyridyl moiety, the number and position of chlorine substitutions on this ring system, as well as N-pyridyl methylation, affected activity.

Cephapirin, with the nitrogen in the 4-position, was 4- and 6-times more active than its 3-pyridyl (BL-P 1343) and 2-pyridyl (BL-P 1314) analogues, respectively. N-Methylation of the 3- or 4-pyridyl moiety produced comparably potent compounds (BL-S 226 and 217, respectively) that were twice as active as cephapirin. Thus, the N-methyl-3- and -4-pyridyl analogues were 10- and 2-fold more active, respectively, than their non-methylated counterparts (BL-P 1343 and cephapirin), indicating

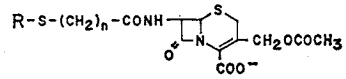
Table 2. Comparative antimycobacterial activity of analogues of cephapirin.



CEPHALOSPORIN	R_1	R_2	MIC * (μg/ml)
CEPHAPIRIN		$-OCOCH_3$	13
BL-P 1343		"	50
BL-P 1314		"	84
BL-S 217		"	5.1
BL-S 226		"	5.3
BL-S 223		"	11
BL-S 187		"	13
BL-S 151		"	1.4
BL-S 190			42
BL-S 119		$-OCOCH_3$	11
BL-S 184		"	11
BL-S 188		"	15
BL-S 189		"	15

* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 4 DETERMINATIONS)

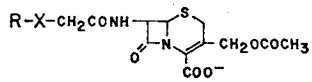
Table 3. Comparative antimycobacterial activity of cephapirin-related homogolues.



CEPHALOSPORIN	R	n	MIC* (μg/ml)
CEPHAPIRIN		1	13
BL-S 224		2	42
BL-P 1343		1	50
BL-S 230		2	84
BL-P 1314		1	84
BL-S 227		2	141
BL-S 151		1	1.4
BL-S 238		2	7.5

* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 4 DETERMINATIONS)

Table 4. Antimycobacterial activity of cephapirin-related compounds having an amino group substituted for the sulfur in the 7-side chain.



CEPHALOSPORIN	R	X	MIC* (μg/ml)
CEPHAPIRIN		-S-	13
BL-S 593		-NH-	3.2
BL-P 1343		-S-	50
BL-S 195		-NH-	7.5
BL-P 1314		-S-	84
BL-S 197		-NH-	35
BL-S 187		-S-	13
BL-S 225		-NH-	15
BL-S 151		-S-	1.4
BL-S 433		-NH-	84

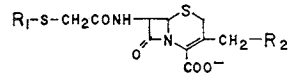
* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 4 DETERMINATIONS)

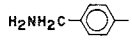
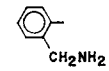
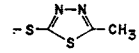
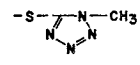
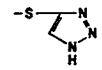
that the effect of the position of the nitrogen in this ring system on activity may be attenuated when this atom is methylated. N-Pyridyl ethylation of cephapirin had no effect on its activity.

As a rule, chlorination and iodation of cephapirin did not appreciably affect its activity. However, its 2, 6-dichloro-substituted analogue (BL-S 151) proved to be a remarkable exception. This derivative was 10-times more active than cephapirin and only 2-fold less active than dihydrostreptomycin. Replacement of the 3-acetoxy of BL-S 151 with a 1-pyridyl substituent caused a substantial loss of activity (BL-S 190).

The relative antimycobacterial activity of a group of cephapirin related homologues wherein the acetamido moiety in the 7-side chain has been replaced with a propionamido group is shown in Table 3. This modification of the 7-side chain resulted in a singnificant reduction of activity. The propionamido homologues (BL-S 224, 227, 230, 238) were 2~5-times less active than the corresponding acetamido homologues. However, as in the acetamido series, the propionamido homologue with the nitrogen in the 4-pyridyl position (BL-S 224) was significantly more active than its 3-pyridyl

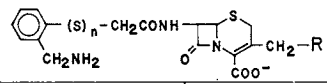
Table 5. Antimycobacterial activity of 7-(aminomethylphenyl-thioacetamido) cephalosporanic acids and 3-(heterocyclic-thiomethyl) substituted analogues.

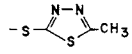
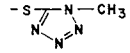
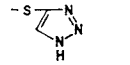


CEPHALOSPORIN	R_1	R_2	MIC* (μg/ml)
MR-S 26		$-OCOCH_3$	71
MR-S 46		"	13
MR-S 37		"	8.9
MR-S 71	"		11
MR-S 72	"		9.5
BL-S 435	"		13

* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 4 DETERMINATIONS)

Table 6. Comparative antimycobacterial activity of 7-[(*o*-aminomethylphenyl) acetamido/thioacetamido] and 3-[(heterocyclic) thiomethyl] substituted cephalosporins.



CEPHALOSPORIN	R	n	MIC* (μg/ml)
MR-S 93	$-OCOCH_3$	0	3.2
MR-S 37		1	8.9
MR-S 96		0	2.7
MR-S 71		1	11
MR-S 94		0	3.2
MR-S 72		1	9.5
BL-S 476		0	3.2
BL-S 435		1	13

* MINIMAL INHIBITORY CONCENTRATION (GEOMETRIC MEAN OF 4 DETERMINATIONS)

congener (BL-S 230) which in turn was more active than the 2-pyridyl analogue (BL-S 227). Although BL-S 238, the 7-(2, 6-dichloropyrid-4-ylthiopropionamido) derivative, was 6-times more active than its unsubstituted parent (BL-S 224), it was still only one-sixth as active as its corresponding acetamido homologue, BL-S 151.

The data in Table 4 show the antimycobacterial activity of a group of cephapirin related compounds having an amino group substituted for the sulfur atom in the 7-side chain. It can be seen that in the aminoacetamido series, just as was previously noted for the thioacetamido series, both the position of the nitrogen in the pyridine ring as well as chlorination of this moiety have an affect on activity. The non-substituted pyridylaminoacetamido derivatives are significantly more active (2~7 times) than their counterparts in the pyridylthioacetamido series. The pyridylamino-

acetamido cephalosporin with the nitrogen in the 4-position (BL-S 593) was 2-fold more active than its 3-pyridyl (BL-S 195) and 10-fold more active than its 2-pyridyl (BL-S 197) analogues. Furthermore, it was 4-times more active than cephapirin. However, unlike cephapirin, chlorination of this derivative was detrimental to its activity. The monochloro-substituted analogue (BL-S 225) was 5 times less active than the parent compound, while the 2, 6-dichloro-substituted derivative (BL-S 433) was only 1/26 as active.

Two compounds that were examined but which are not included in the tables are BL-S 192, 7-(4-amino-pyrid-1-ylacetamido) cephalosporanic acid, and BL-S 194, 7-(phenylaminoacetamido) cephalosporanic acid. The former compound was found to be as active as BL-S 593 (MIC of 3.2 μg/ml), the 7-(pyrid-4-ylaminoacetamid) derivative, whereas the latter compound was poorly active (MIC of 100μg/ml) indicating the requirement of a pyridine ring for antimycobacterial activity in the aminoacetamido series.

Significant antimycobacterial activity was also found for a series of semisynthetic cephalosporins that did not have a pyridine or a substituted pyridine in the 7-side chain. The data in Table 5 show the relative potency of 7-(phenylthioacetamido) cephalosporins that have an aminomethyl substituent in the ortho, meta, or para position. The position of this group on the phenyl ring, just as was observed with the nitrogen in the pyridine ring, affected activity, except in reverse order. Here, MR-S 37, the ortho-aminomethyl substituted analogue, was almost twice as active as the compound with the meta-substituent (MR-S 46), and 8-times more active than the para-substituted analogue (MR-S 26). When heterocyclic-thiomethyl groups (MR-S 71, 72, and BL-S 435) were substituted for the 3-acetoxymethyl group of the *o*-aminomethyl-substituted cephalosporin (MR-S 37), antimycobacterial activity was unaffected.

The activity of 7-[(*o*-aminomethylphenyl) acetamido] cephalosporins in which a sulfur bridge is not present in the 7-side chain was significantly different from that obtained with compounds containing this atom as shown by the data in Table 6. MR-S 93, the 7-[(*o*-aminomethylphenyl) acetamido] cephalosporin, as well as its 3-(heterocyclic-thiomethyl)-substituted derivatives (MR-S 94, 96, and BL-S 476), were 3$\sim$4 times more active than the corresponding thioacetamido compounds (MR-S 37, 71, 72, and BL-S 435). In the 7-[(*o*-aminomethylphenyl) acetamido] series of compounds, just as was previously noted for the thioacetamido derivatives, when heterocyclic-thiomethyl groups were substituted for the 3-acetoxymethyl moiety, activity was unaffected.

The significant contribution of the aminomethyl substitutent to the activity of MR-S 93 was indicated from results obtained with its unsubstituted analogue, BL-S 271, 7-(phenylacetamido) cephalosporanic acid. This compound (MIC of 100 μg/ml) was only 1/30 as active as MR-S 93.

In summary, more than 600 semisynthetic cephalosporins have been screened for activity *in vitro* against *M. tuberculosis* H37Rv. Most of the compounds, including approximately 130 7-(α-amino-α-phenylacetamido) derivatives, did not demonstrate activity superior to that of 7-ACA.

Compouds having a pyridylthio- or pyridylamino-acetamido 7-side chain were found to be quite active. Within this series, activity could be affected by altering the position of the nitrogen in the pyridyl ring, as well as by appropriate chlorination or N-methylation of this moiety. Activity was also found to vary depending upon whether the 7-side chain contained a sulfur or an amino bridge. Antimycobacterial activity was higher when the nitrogen was in the 4-position in the pyridylthio- or pyridylamino moiety and even further improved when the pyrid-4-ylthio derivative was chlorinated in the 2- and 6-positions. In fact, the most active compound *in vitro* was a

chlorinated analogue of cephapirin, BL-S 151, 7-(2, 6-dichloropyrid-4-ylthioacetamido) cephalosporanic acid, which was only 2-fold less active than dihydrostreptomycin. The most active derivative in the pyridylamino series, BL-S 593 (pyrid-4-ylaminoacetamido), and an equally potent but an unrelated pyridyl-containing compound, BL-S 192 (4-aminopyrid-1-ylacetamido), were only 2-fold less active than BL-S 151. In addition to these derivatives, a high degree of activity was also found among a series of compounds having an aminomethylphenyl- or aminomethylphenylthio-acetamido group at the 7-position. Activity was affected by the position of the aminomethyl substituent, and by the presence or absence of the sulfur bridge. The most active derivatives (MR-S 93, 94, 96, and BL-S 476), which were about one-fourth as active as dihydrostreptomycin, had an *o*-aminomethyl substituent, and lacked the sulfur bridge.

In vivo studies were carried out in mice experimentally infected with *M. tuberculosis* H37Rv. The compounds tested were BL-S 151, 217, 192, and a BL-S 192 derivative. BL-S 151 and 217 were ineffective, whereas the other two significantly prolonged the survival of infected mice.

It is hoped that this presentation will stimulate interest in investigating the potential utility of cephalosporins as antitubercular antibiotics.

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