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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF LIPOPHILIC CARBAPENEMS WITH ANTI-MRSA ACTIVITY

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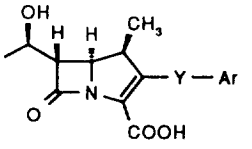
Abstract: A series of sulphur-linked and carbon-linked lipophilic carbapenems has been prepared and evaluated for antibacterial activity *in vitro* and *in vivo* and for affinity for PBP2' of *Staphylococcus aureus*. Potent activity *in vitro* against methicillin-resistant *S. aureus* and methicillin-resistant coagulase negative staphylococci was observed despite IC_{50} values for PBP2' being higher than the MIC.

The evolution of antibiotic resistance among Gram-positive pathogens is a world-wide phenomenon of great concern especially in hospitals. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (MRCNS) have increased dramatically in recent years. Since few antibiotics are available for the treatment of these infections and those that are have limited use because of adverse effects, potent new antimicrobial agents are desirable [(1)].

Carbapenem antibiotics, for example imipenem and meropenem, possess an extremely broad spectrum of activity and are used clinically for the treatment of severe infections; however, they lack utility against methicillin resistant staphylococci and penicillin resistant enterococci because they have low affinity for the penicillin binding proteins of these organisms [(2)]. Introduction of a lipophilic moiety at the C-3 position of a cephalosporin [(3)] or carbacephalosporin [(4)], or at the C-2 position of a penem [(5)] or carbapenem [(6)] confers potency against MRSA in particular.

In a programme of research aimed at the discovery of new antibacterial agents with activity against MRSA, we synthesised a series of carbapenems with a lipophilic aromatic moiety attached by various links to the C-2 position.



Y = S or S-X or CH_2X

We report the results obtained in the S, S-X and CH_2-X-linked series. Antibacterial activity and affinity for PBP2' were measured and compared to imipenem and L695256 [(7)], and efficacy *in vivo* evaluated; the observed SAR are discussed.

Synthesis [(8)]:

S linked series (Y = S or SX): The general synthesis used the displacement of a C-2-diphenylphosphate by nucleophilic thiols [(9)] and subsequent deprotection (Scheme 1).

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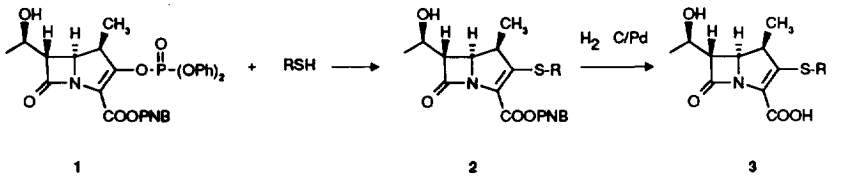
Scheme 1

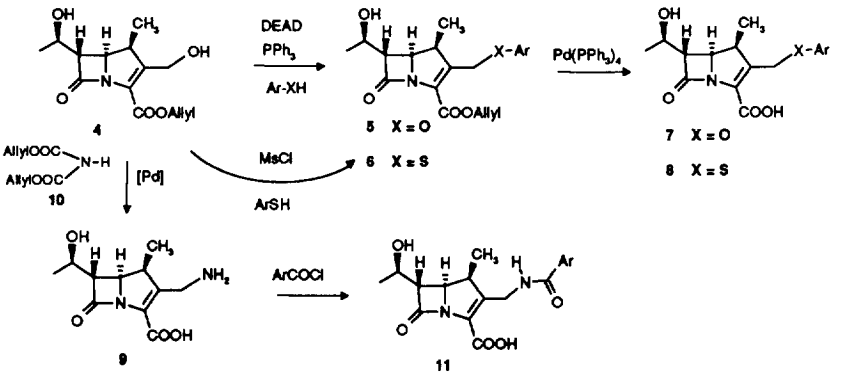


1 2 3

Y = CH_2-X series : The synthetic approach to CH_2X-Ar carbapenems **7** and **8** was based on the functionalisation of 2-hydroxymethylcarbapenem intermediate **4** [10] (Scheme 2). CH_2O derivatives **5** were obtained by reaction of **4** with corresponding phenols under Mitsunobu conditions (DEAD-PPh_3, toluene). With the exception of **6b** and **6d**, CH_2S analogues **6** were prepared by the same method (ADDP-Bu_3P, toluene); **6b** and **6d** were prepared by conversion of **4** into mesylate [11] (MsCl, Et_3N, 0°C, CH_2Cl_2), and reaction with the corresponding thiols. The allyl protection was removed by treatment with $Pd(PPh_3)_4$ in the presence of sodium 2-ethylhexanoic acid [12]. Subsequent purification on a reverse phase C18 column provided the carbapenems **7**, **8**.

Reaction of **4** with aminating agent **10** under Mitsunobu conditions [10] and subsequent palladium deprotection gave 2-aminomethylcarbapenem intermediate **9**, converted to the amidic derivative **11** by reaction with 2-naphthoic acid chloride.

Scheme 2



Biological properties :

The influence of the linking group on the antibacterial activity was investigated using naphthalene as the aromatic unit. *In vitro* activity against Gram-positive bacteria was determined by an agar dilution susceptibility test [13] and the data are given in Table 2. Against methicillin sensitive *S. aureus*, all carbapenems with the exception of **11** were at least as potent as imipenem, but potency against MRSA or MRCNS depended on the nature of the link. S, SCH_2, CH_2O, and CH_2S links gave compounds (**3a, 3b, 7a, 8a**) with a range of potencies (MICs ≤ 8µg/mL) with **3b** as active as L695256. In contrast, SCH_2CH_2, $S(CH_2)_2NHCO$ or CH_2NHCO (**3c, 3d and 11**) had deleterious effects on activity with MICs no better than those of imipenem. In general the presence of an amide or amine function (**3d, 11**) in the linking group led to poor antibacterial activity.

Optimal growth of *Streptococcus pneumoniae* requires the addition of 5% horse blood to the test medium but the activity of some compounds was severely compromised by its presence; *S. aureus* 601055 was included in the tested plates with and without blood to control for this effect. For CH_2O and CH_2S analogues, MICs increased from 0.016 to 32 µg/ml in the presence of blood suggesting that the compounds may be more potent

against *S. pneumoniae* and enterococci than the MICs under these conditions predicted. The nature of this effect has not been clarified but the degree of loss of activity in the presence of blood could not be accounted for by enzymatic degradation of compound (there was no difference in loss of activity in the presence of 5% heat-denatured blood) nor binding to serum protein (binding to 5% blood was <40%).

Table 1. Carbapenems prepared :Y-Ar variation

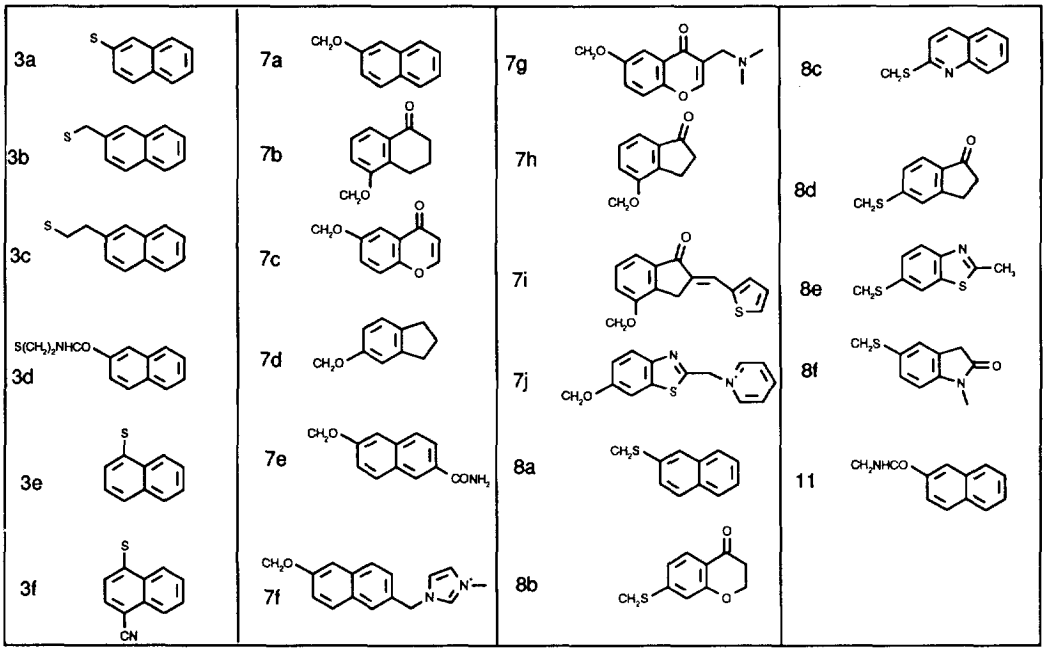


Table 2. Antibacterial activity: influence of the link

MIC (μg/ml)

	Link	S	SCH_2	CH_2O	CH_2S	SCH_2CH_2	$S(CH_2)_2NHCO$	CH_2NHCO		
Organism		**3a**	**3b**	**7a**	**8a**	**3c**	**3d**	**11**	L695256	imipenem
S. aureus	601055 MS	0.03	0.06	0.016	0.016	0.13	0.13	0.5	0.03	0.03
	601053 MR	1	1	0.25	0.5	8	4	8	0.5	8
	607004 MR	8	1	4	4	32	64	128	1	64
S. haemolyticus	601117 MR	8	2	2	2	128	256	128	2	64
S. aureus *	601055 MS	0.03	0.03	32	32	0.13	0.13	0.25	0.03	0.015
S. pneumoniae	671319 penR	2	0.06	4	1	0.5	0.25	4	0.015	0.12
E. faecalis	683026 vanS	16	2	128	128	8	4	16	0.13	2
E. faecium	628016 vanR	8	8	64	128	16	8	32	2	4

* grown in the presence of blood

To determine the influence of the aromatic unit on antibacterial activity, a series of compounds was prepared with various bicyclic aromatic moieties and CH_2O or CH_2S as the linking group. Replacement of naphthalene by more hydrophilic systems eliminated the effect of blood on antimicrobial activity, apart from the indane derivative **7d** (Table 3). With the exception of **7d** and **8f** the analogues in Table 3 were broadly as potent as the naphthalene derivatives **7a** and **8a** against MRSA and MRCNS but were clearly superior against streptococci and enterococci.

Table 3. Antibacterial activity : Influence of the aromatic group

MIC (μg/ml)

	link	CH_2O				CH_2S					
Organism		**7a**	**7b**	**7c**	**7d**	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**
S. aureus	601055 MS	0.016	0.016	0.016	0.03	0.016	0.03	0.016	0.016	0.03	0.12
	601053 MR	0.25	0.25	0.25	1	0.5	1	0.13	0.5	2	4
	607004 MR	4	2	2	16	4	1	4	2	4	8
S. haemolyticus	601117 MR	2	4	2	16	2	1	0.5	2	4	16
S. aureus *	601055 MS	32	0.008	0.016	8	32	0.008	0.03	0.008	0.016	0.06
S. pneumoniae	671319 penR	4	0.06	0.03	1	1	0.03	0.06	0.03	0.06	0.25
E. faecalis	683026 vanS	256	2	2	64	128	1	4	1	2	4
E. faecium	628016 vanR	64	2	2	32	128	0.5	1	0.5	8	8

* grown in the presence of blood

Substitution of the naphthalene ring by $CONH_2$ or methylimidazolium group (Table 4) resulted in an improvement in the *in vitro* activity against streptococci and enterococci (**7e, 7f** compared to **7a**).

In the chromone series, the introduction of a basic group (**7g**) improved activity against enterococci, an effect similar to that seen with the pyridine substituted benzothiazole (**7j**). We also noted that the activity against enterococci correlated with the overall hydrophilicity of the molecule (data not shown).

In the S-linked series, para cyano substitution (**3f**) led to improved potency against a homogenous methicillin resistant *S. aureus* (MR1, data not shown), an effect which was also demonstrated by the introduction of a methylenethienyl group on the tetralone nucleus (**7i**). Potency against the homogenous MRSA, MR1 was assumed to compare well with the affinity of the compound for PBP2' [14] (but see below for exceptions).

Table 4. Antibacterial activity : influence of the substitution

MIC (μg/ml)

Organism		**7a**	**7e**	**7f**	**7c**	**7g**	**3e**	**3f**	**7h**	**7i**	**8e**	**7j**
S. aureus	601055 MS	0.016	0.008	0.016	0.016	0.06	0.06	0.016	0.016	0.008	0.03	0.03
	601053 MR	0.25	0.25	0.5	0.25	0.5	1	0.5	0.12	0.25	2	0.25
	607004 MR	4	0.5	1	2	4	8	2	4	2	4	4
S. haemolyticus	601117 MR	2	2	2	2	2	8	1	8	0.5	4	2
S. aureus *	601055 MS	32	0.016	0.016	0.016	0.016	0.06	0.016	0.008	0.008	0.016	0.016
S. pneumoniae	671319 penR	4	0.03	0.06	0.03	0.12	1	0.25	0.06	0.03	0.06	0.06
E. faecalis	683026 vanS	256	2	2	2	0.5	16	8	1	2	2	0.5
E. faecium	628016 vanR	64	1	2	2	2	32	2	2	4	8	2

* grown in the presence of blood

The synthetic program produced compounds with a range of *in vitro* activities. **7e** and **7f** had the best overall activity, equivalent to L695256 with MICs against staphylococci ≤2μg/mL. Against penicillin resistant *S. pneumoniae* potency was better than imipenem and equivalent to L695256, although it was not possible to improve on the activity of imipenem against enterococci.

Compounds **3b**, **7e**, **7f** and **7g** were evaluated in a thigh infection model in a mouse [15]. Compounds **7e** and **7g** reduced bacterial numbers below the infecting inoculum of MSSA at a dose of 2mg/kg and against methicillin resistant clinical isolate (065001) at a dose of 5mg/kg. The majority of the carbapenems tested were highly protein bound (> 90 %) with the exception of **7f** (80%) which incorporated the methylimidazolium substituent. There did not appear to be a relation between *in vivo* activity and the level of affinity of the molecule for plasma proteins as the most efficacious compounds, **7e** and **7g**, were, respectively, 98.1% and 95.7% bound to mouse serum protein.

Binding to PBP2'

Carbapenems with activity against MRS are hypothesised to have higher affinity for PBP2′ than for example imipenem[16]. The affinity of **3b** and **7e** for PBP2' was measured by a method based on that of Brown and Reynolds [17] and compared with imipenem and L695256 [16]. Both **3b** and **7e** saturated PBP2' at lower concentrations than imipenem, but IC_{50} values were not as low as the MIC (Table 5). In contrast, the IC_{50} for L695256 was approximately the same as its MIC.

Table 5. Binding of carbapenems to PBP2'.

	IC_{50} μg/mL		MIC for homogenous MRSA
	this study [16]	literature	MR1 μg/mL
Imipenem	430	220 [18]	128
L695256	1.3	0.4-1.8 [16]	2
3b	9.5	-	2
7e	>16*	-	8

*Measured by the intact-cell-based method[17] only

Conclusion :

We have synthesised a new series of lipophilic carbapenems with a lipophilic moiety attached to the carbapenem nucleus by various linking groups. We have shown that the antibacterial activity was greatly influenced by the nature of the link and the nature of the aromatic unit. We have found compounds which exhibit good *in vitro* and *in vivo* activity against Gram-positive strains intrinsically less susceptible to existing carbapenems.

Acknowledgements

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