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DICATIONIC 2-FLUORENONYLCARBAPENEMS: POTENT ANTI-MRS AGENTS WITH IMPROVED SOLUBILITY AND PHARMACOKINETIC PROPERTIES

Mark L. Greenlee,[a]* Joanne B. Laub,[a] Gregory P. Rouen,[a] Frank DiNinno,[a] Milton L. Hammond,[a] Joann L. Huber,[b] Jon G. Sundelof,[b] and Gail G. Hammond[c]

Departments of [a]Medicinal Chemistry, [b]Infectious Disease, and [c]Biochemistry
Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065, U.S.A.

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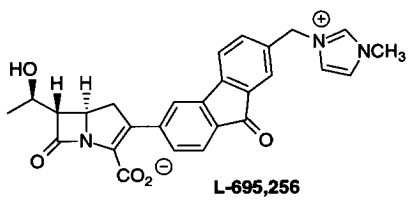
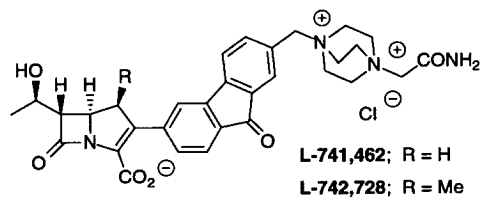
Abstract: The synthesis and biological evaluation of a series of dicationic-substituted 2-fluorenonylcarbapenems is described. This class of compounds showed enhanced water solubility while maintaining potent activity against MRS. Introduction of a 1-β-methyl substituent was found to improve pharmacokinetics.

Introduction: Infections caused by methicillin-resistant staphylococci (MRS) are becoming an increasingly serious problem worldwide.[1] Strains of MRS are generally multiply drug-resistant, including to all currently available β-lactam antibiotics, and thus the therapeutic options for treating these infections are extremely limited.[2] Vancomycin is currently the drug of choice for treating MRS infections, but reports of MRS strains resistant to this antibiotic have started to appear.[3] Recent reports from these laboratories have described the discovery of 2-*meta*-biphenylcarbapenems with excellent activity against MRS.[4] Further refinements led to L-695,256, a 2-fluorenonylcarbapenem with potent activity against MRS both in vitro and in vivo.[5] The enhanced MRS activity of these 2-arylcarbapenems is believed to be due to increased binding to PBP2a, the low affinity penicillin-binding protein which mediates methicillin resistance in staphylococci.[6] In addition, a cationic substituent on the aryl sidechain, as in L-695,256, has been found to be beneficial for in vivo efficacy through a reduction in serum protein binding. However, the development of L-695,256 and related zwitterionic 2-arylcarbapenems has been plagued by low water solubility. This led us to investigate the replacement of the methylimidazolium group of L-695,256 with a dicationic substituent in order to increase hydrophilicity and thereby improve water solubility. Such a compound would also have an associated counterion which could be



L-695,256



L-741,462; R = H
L-742,728; R = Me

varied to modify its physical properties if necessary. After examining several types of dicationic groups, we found that substitution with a dicationic moiety derived from 1,4-diazabicyclo[2.2.2]octane (DABCO) led to compounds with particularly favorable properties. Members of the resulting class of cationic zwitterions showed generally enhanced water solubility while maintaining good activity against MRS. Both these properties were, however, influenced by the nature of the DABCO substituent. A study of the structure-activity relationships led to the identification of L-741,462 as a promising candidate with excellent water solubility and activity against MRS approximately equal to that of vancomycin. The introduction of a 1-β-methyl substituent to give L-742,728 was also investigated,[7,8] and led to significantly improved pharmacokinetic parameters.

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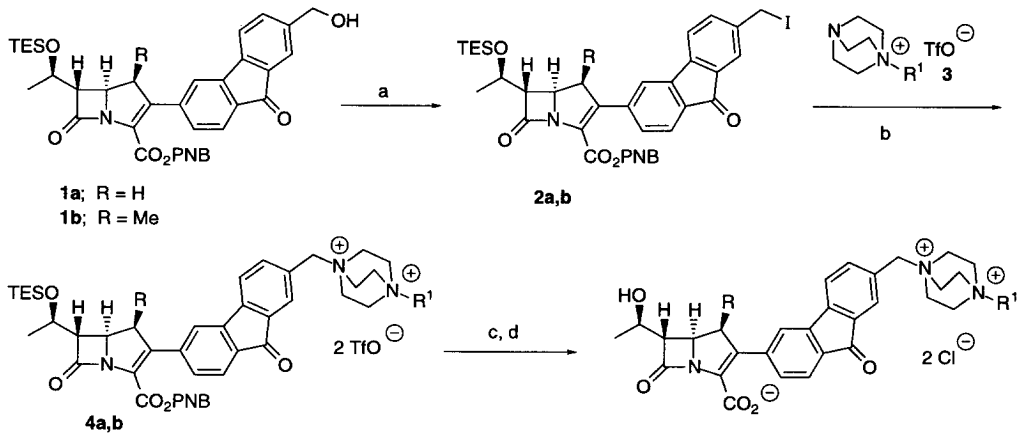
Chemistry: The protected 2-fluorenonylcarbapenem intermediates **1a** and **1b** were synthesized by the Suzuki cross-coupling method of Yasuda et. al.[9] Introduction of the dicationic substituent was accomplished by an activation-displacement process (Scheme 1). The alcohol **1a** was converted to the benzylic iodide **2a** by mesylation followed by treatment with sodium iodide. Reaction of **2a** with a variety of mono-quaternized DABCO reagents **3**[10] was promoted with silver triflate to provide the diammonium bis-triflate salts **4a**. Silver triflate promotion was found to give a cleaner, more rapid reaction than direct displacement of the iodide. As an added advantage, the bis-triflate salts **4a** often were highly crystalline and could be isolated in pure form by precipitation with diethyl ether. The corresponding 1-β-methylcarbapenem analogs **4b** were synthesized in an exactly analogous manner starting with intermediate **1b** in Scheme 1. Removal of the protecting groups from

Scheme 1

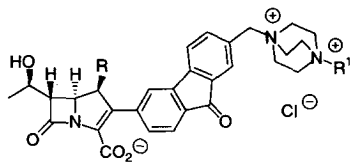


(a) i. MsCl, Et_3N, CH_2Cl_2, -20 °C; ii. NaI, acetone (90-95%); (b) AgOTf, CH_3CN (75-95%); (c) i. HCl, H_2O, THF; ii. H_2, Rh/C, pH 7; (d) i. NaCl, H_2O; ii. Reverse phase MPLC.

4a or **4b** was accomplished in a one pot sequence. The triethylsilyl ether was first hydrolyzed in acidic aqueous THF (~pH 2.3) at 0 °C. After adjusting to pH 7 with MOPS buffer, the *p*-nitrobenzyl ester was removed by hydrogenolysis at atmospheric pressure over rhodium on carbon. The use of rhodium on carbon as catalyst minimized reductive cleavage of the benzylic diammonium group, which was a significant side-reaction when palladium on carbon was employed. Purification was accomplished by reverse phase MPLC on Amberchrom® CG-1000 polystyrene resin to give the carbapenem antibiotics (see Tables 1–2).

Biological Evaluation: Table 1 presents the in vitro antibacterial activity of a variety of DABCO-based dicationic 2-fluorenonylcarbapenems evaluated against panels of clinically relevant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCNS). The prototype **5** (R^1 = Me) was less active against MRSA than L-695,256, but was equipotent versus the MRCNS panel. Structure-activity studies resulted in improvement in activity as the DABCO substituent R^1 was extended or functionalized. The best compound in this initial series was the carbamoyl-methyl substituted analog L-741,462, which showed increased activity against both MRSA (0.5x vancomycin) and MRCNS (2x vancomycin) and was highly soluble in water (>100 mg/mL).

Table 1. Anti-MRSA/MRCNS Activity and PBP2a Binding



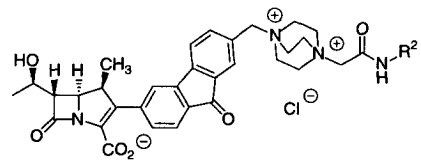
Compd	R	R^1	MRSA (n=15)[a] Range	MRSA (n=15)[a] MIC_{50}	MRSA (n=15)[a] MIC_{90}	MRCNS (n=9)[a] Range	MRCNS (n=9)[a] MIC_{50}	MRCNS (n=9)[a] MIC_{90}	PBP2a IC_{50}[b]
5	H	Me	1–8	2	8	2–4	2	4	0.18
6	H	Et	1–8	2	4	1–4	2	4	0.25
7	H	*n*-Pr	0.5–4	2	4	1–4	2	4	0.28
8	H	CH_2CH_2OH	0.5–8	2	4	1–4	2	4	0.17
9	H	$CH_2CH_2CH_2OH$	1–8	2	8	1–4	4	4	0.80
10	H	$CH_2CO_2CH_3$	2–32	8	16	4–32	16	16	--
11	H	CH_2COCH_3	1–8	2	8	2–8	4	4	--
12	H	CH_2COPh	0.5–4	2	4	0.5–4	2	4	--
13	H	CH_2CN	0.5–4	2	4	1–4	2	4	0.29
L-741,462	H	CH_2CONH_2	0.5–4	1	4	1–4	2	2	0.34
14	Me	Me	1–4	2	4	2–8	4	8	0.74
15	Me	*n*-Pr	1–8	4	4	2–8	4	8	0.57
16	Me	CH_2CH_2OH	1–4	4	4	2–16	4	8	0.65
17	Me	$CH_2CH_2CH_2OH$	1–4	2	4	2–16	4	8	0.47
18	Me	CH_2CN	0.5–8	2	4	2–8	4	8	0.29
19	Me	CH_2Ph	0.25–4	1	2	1–8	2	8	2.2
20	Me	CH_2COPh	0.25–4	1	2	1–8	2	4	0.62
21	Me	CH_2CH_2OPh	0.25–2	1	2	0.5–4	2	4	0.40
22	Me	$(CH_2)_3CONH_2$	1–8	4	4	2–16	4	8	2.0
L-742,728	Me	CH_2CONH_2	1–4	4	4	2–8	4	8	0.80
23	H	$CH_2CONHMe$	1–8	2	8	1–4	2	4	--
24	H	CH_2CONMe_2	1–8	4	4	1–4	4	4	--
25	H	$CH_2CONHPh$	0.063–2	0.5	2	0.25–2	1	1	0.33
26	Me	$CH_2CONHMe$	1–8	4	8	2–8	4	8	0.33
27	Me	CH_2CONMe_2	2–8	4	8	2–16	4	16	0.39
28	Me	$CH_2CONHPh$	0.5–2	2	2	0.5–4	2	4	1.28
29	Me	$CH_2CON(Me)Ph$	1–4	4	4	2–16	8	16	0.90
30	Me	$CH_2CONH(n\text{-}Pr)$	1–4	2	4	2–8	4	8	0.84
31	Me	$CH_2CONH(i\text{-}Pr)$	0.5–4	2	4	1–8	4	8	0.85
32	Me	CH_2CONH(cyclopentyl)	0.5–4	2	4	2–8	4	8	1.0
33	Me	$CH_2CONHt\text{-}Bu$	0.5–4	2	4	1–8	4	8	2.4
34	Me	$CH_2CONHCH_2Ph$	0.13–2	1	2	0.5–4	1	4	0.97
L-695,256			0.25–4	1	2	0.5–4	2	4	0.5
Imipenem[c]			1–128	64	128	32–256	128	256	100
Vancomycin[c]			1–4	1	2	2–8	4	4	--

(a) MICs (μg/mL) were determined using the broth microdilution method. Mueller-Hinton broth + 2% NaCl, inoculum ~10^5 cfu/mL, incubation at 35 °C for 46 h. MICs read to no visible growth. (b) Binding to PBP2a (μg/mL) was evaluated by competition analysis with [^{3}H]-benzylpenicillin using cell membrane fractions from the MRSA COL strain. (c) Antibacterial data for imipenem and vancomycin reflect the mode of twenty-nine measurements of each panel of strains.

Introduction of a 1-β-methyl group was found to have little effect on MRSA activity, but generally resulted in a reduction in activity against MRCNS. This trend is illustrated by comparing L-741,462 with its 1-β-methyl counterpart L-742,728. However, this decrease in activity was offset by a substantial improvement in pharmacokinetic parameters (vide infra). A number of substituted amide derivatives of compounds L-741,462 and L-742,728 were prepared (**23–34**, Table 1), with an emphasis on the 1-β-methyl series based on its

pharmacokinetic advantage. Among these analogs, the phenyl amides **25** and **28** stood out by virtue of their excellent MRS activity. Unfortunately, the presence of an additional hydrophobic moiety in these compounds resulted in an unacceptable decrease in water solubility. This effect was also observed with certain other hydrophobic analogs (**12**, **19**, **21** and **34**), while the majority of the compounds in Table 1 showed good solubility in water (>20 mg/mL). Introduction of polar substituents on the phenyl ring of **28** and replacement of the phenyl with a heteroaryl group were both explored, but did not restore adequate aqueous solubility (Table 2). However, further increases in activity were achieved: the most potent compound, the 4-carbamoyl substituted analog **48**, was four-fold more active than vancomycin against MRSA and twice as active against MRCNS. Consistent with the good MRS activity of the compounds in Tables 1–2, all were found to bind to PBP2a with high affinity (IC_{50} <2.5 μg/mL). However, small variations in MRS activity do not correlate with PBP2a IC_{50} values, suggesting that other factors are also important in determining exact levels of potency.

Table 2. Anti-MRSA/MRCNS Activity and PBP2a Binding



Compd	R^2	MRSA (n=15)[a] Range	MRSA (n=15)[a] MIC_{50}	MRSA (n=15)[a] MIC_{90}	MRCNS (n=9)[a] Range	MRCNS (n=9)[a] MIC_{50}	MRCNS (n=9)[a] MIC_{90}	PBP2a IC_{50}[b]
35	2-pyridyl	0.25–4	2	4	1–8	2	8	0.31
36	3-pyridyl	0.25–4	1	2	1–8	2	4	0.44
37	4-pyridyl	0.25–2	1	2	1–8	2	4	0.86
38	2-thiazoyl	0.5–4	2	4	2–16	4	8	0.62
39	3-$HOCH_2$-Ph	0.13–4	1	2	0.5–4	1	4	1.2
40	4-$HOCH_2$-Ph	0.25–2	1	2	0.25–4	1	4	2.0
41	4-($HOCH_2CH_2$)-Ph	0.25–2	1	2	0.5–8	2	4	1.0
42	2-MeO-Ph	0.25–2	1	2	1–8	2	4	0.65
43	3-MeO-Ph	0.13–2	1	1	0.25–4	1	4	1.82
44	4-MeO-Ph	0.13–2	1	1	0.5–4	1	4	0.89
45	4-HO-Ph	≤0.03–1	0.5	1	0.5–4	1	4	0.48
46	2-(H_2NCO)-Ph	0.25–4	2	4	1–16	2	8	0.78
47	3-(H_2NCO)-Ph	0.13–2	1	1	0.5–4	1	2	0.93
48	4-(H_2NCO)-Ph	≤0.03–0.5	0.25	0.5	0.13–2	0.5	2	1.5
49	2-(NC)-Ph	0.25–2	1	2	2–8	2	4	0.82
50	3-(NC)-Ph	≤0.03–1	0.5	1	0.25–2	1	2	0.56
51	4-(NC)-Ph	0.06–2	1	2	0.5–4	1	4	1.05
52	3-(MeCO)-Ph	0.06–2	1	2	0.5–4	1	4	0.98
53	4-(MeCO)-Ph	≤0.03–2	0.5	1	0.25–4	1	4	2.4
	L-695,256	0.25–4	1	2	0.5–4	2	4	0.5
	Imipenem[c]	1–128	64	128	32–256	128	256	100
	Vancomycin[c]	1–4	1	2	2–8	4	4	--

For footnotes a, b, c, see Table 1.

The in vitro antibacterial activity of L-741,462 and L-742,728 against a range of gram-positive and gram-negative bacteria is shown in Table 3. In addition to excellent activity against MRS, both compounds were also more active than imipenem against vancomycin-resistant enterococcal strains and penicillin-resistant *S. pneumoniae*, albeit less so than L-695,256. While primarily active against gram-positive bacteria, these

dicationic 2-fluorenonylcarbapenems also demonstrated significant activity against a panel of thirteen gram-negative organisms selected from eight enteric genera. As expected for 2-arylcarbapenems,[8] both compounds were substantially less susceptible to the mammalian dehydropeptidase, DHP-I, than imipenem.

In a mouse systemic infection model, L-741,462 and L-742,728 were as efficacious as imipenem and L-695,256 against methicillin-susceptible *S. aureus* (MSSA). Against an MRSA infection, the two cationic zwitterions were both more active than vancomycin and comparable in activity to L-695,256 (Table 4). The pharmacokinetic parameters for L-741,462 and L-742,728 in rhesus monkeys are compared in Table 5. The 1-β-methyl compound showed significantly better performance with a slower rate of clearance from the plasma, an extended terminal half-life and a substantially higher urinary recovery. The pharmacokinetic performance of L-742,728 was also superior to that of L-695,256 and imipenem.[11]

Table 3. Antibacterial Spectrum of L-741,462 and L-742,728

Species (No.)	MIC (μg/mL)[a] L-741,462	L-742,728	L-695,256	Imipenem	Vancomycin
MSSA (4)	0.03	0.03	0.03	0.03	1
E. faecalis (1)	0.125	0.5	0.125	2	4
VanR *E. faecalis* (1)	2	8	1	8	32
VanR *E. faecium* (1)	16	8	4	>64	>64
S. pyogenes (1)	≤0.031	≤0.031	≤0.031	≤0.031	0.25
PenR *S. pneumoniae* (2)	0.09	0.24	≤0.03	0.35	0.25
Gram Negative (13)	1.05	3.6	1.37	0.45	>64
DHP-I Susceptibility[b]	0.055	0.021	0.078	1.0	--

(a) Broth microtube dilution method. Tested in Mueller-Hinton broth (MHB) at ~10^5 cfu/mL with the following exceptions: Enterococci tested in brain heart infusion broth at ~10^6 cfu/mL; Streptococcus tested in MHB + 2.5% lysed horse blood. Incubation at 35 °C for 20–22 h. Activity is reported as the geometric mean of the MICs for the number of strains indicated. (b) DHP-I (porcine). Rate of hydrolysis relative to imipenem as substrate.

Table 4. In Vivo Activity in a Mouse Systemic Infection Model

		L-741,462	L-742,728	L-695,256	Vanco	Imipenem
MSSA[a]	MIC (μg/mL)	0.004	0.031	0.008	1	0.008
	ED_{50} (mg/kg)	0.042	0.021	0.034	3.7	0.021
MRSA[b]	MIC (μg/mL)	1	2	1	1	64
	ED_{50} (mg/kg/dose)	0.5	1	0.92	2.33	15.7

(a) Charles River CD-1 female mice. Infecting organism *S. aureus* MB2985. Challenge dose ~4x10^7 cfu IP in brain heart broth. Antibiotics administered SC at 0 h. (b) Taconic Farms DBA/2 female mice. Infecting organism MR *S. aureus* 76 Virginia. Challenge dose ~1x10^7 cfu IP in 5% hog gastric mucin. Antibiotics administered SC at 0 and +4 h.

Table 5. Pharmacokinetic Parameters in Rhesus Monkeys[a]

		L-741,462	L-742,728	L-695,256 (n=3)	IPM/CIL[b]
Plasma Clearance	(mL/min/kg)	4.74	2.95	5.56 ± 0.76	6.72
Renal Clearance	(mL/min/kg)	1.88	2.72	2.34 ± 0.81	5.44
GFR	(mL/min/kg)	2.96	3.48	3.19 ± 0.51	2.98
AUC, 0 – ∞	(μg • h/mL)	35.14	56.44	30.4 ± 4.5	24.79
Urinary Recovery	(0 – ∞, %)	41	87	41 ± 11	82
β $t_{1/2}$	(h)	0.91	1.35	1.17 ± 0.14	0.60

(a) Dose = 10 mg/kg by IV bolus injection. Compounds compared in direct cross-over experiments in a ~4.6 kg rhesus monkey except for L-695,256, for which the mean values and standard deviation from data obtained with three monkeys (3.7–7.4 kg) are given. For methodology see ref 11. (b) Imipenem/Cilastatin.

Conclusions: A new class of water soluble MRS-active 2-fluorenonylcarbapenems based on a DABCO dicationic motif has been discovered. Structure-activity studies led to the identification of L-742,728, which was found to have activity against MRS comparable to that of vancomycin both in vitro and in vivo, significantly improved pharmacokinetic properties and high water solubility in both amorphous and crystalline forms. While the development of L-742,728 has not been pursued due to an immune-based toxicity,[12] the lessons that have been learned in this study should aid in the ultimate development of carbapenems[13] or other β-lactam antibiotics for the treatment of infections due to MRS.

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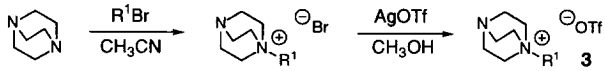
10. Reagents **3** were readily synthesized as shown below by alkylation of DABCO with the requisite alkyl bromide followed by anion exchange with silver triflate.



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