Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 673–678

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND ACTIVITY OF 2-(SULFONAMIDO)METHYL-CARBAPENEMS: DISCOVERY OF A NOVEL, ANTI-MRSA 1,8-NAPHTHOSULTAM PHARMACOPHORE

R. R. Wilkening,*[a] R. W. Ratcliffe,[a] K. J. Wildonger,[a] L. D. Cama,[a] K. D. Dykstra,[a] F. P. DiNinno,[a] T. A. Blizzard,[a] M. L. Hammond,[a] J. V. Heck,[a] K. L. Dorso,[b] E. St. Rose,[b] J. Kohler,[b] and G. G. Hammond[c]

Departments of [a]Medicinal Chemistry, [b]Infectious Disease and [c]Biochemistry, Merck Research Laboratories Merck & Co., Inc., P.O. Box 2000, Rahway, New Jersey, 07065-0900, U.S.A.

Received 24 November 1998; accepted 19 January 1999

Abstract: A series of 1β-methyl carbapenems substituted at the 2-position with lipophilic, acyclic and cyclic (sulfonamido)methyl groups was prepared and evaluated for activity against resistant gram-positive bacteria. From these studies, the 1,8-naphthosultamyl group emerged as a novel, PBP2a-binding, anti-MRSA pharmacophore worthy of further exploration.

Introduction

The last two decades have witnessed a dramatic, worldwide increase in multidrug-resistant strains of common gram-positive bacteria.[1] The most serious examples are methicillin-resistant staphylococci (MRS)[2] and penicillin-resistant pneumococci and enterococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (MRCNS) currently rank among the leading causes of severe infections in both the hospital and community environments. The multidrug resistance of most MRS has often left the glycopeptide antibiotics vancomycin and teicoplanin[3] as the only therapeutic options, and vancomycin use is compromised by adverse side effects and slow clinical response. Moreover, the marked increase in vancomycin use in the 1990s has been associated with the emergence of vancomycin-resistant enterococci (VRE).[4] Since gram-positive cocci are known to share their genetic material, it seems quite likely that vancomycin resistance in enterococci will be transferred[5] to more pathogenic bacteria, especially MRSA, leading to organisms for which there are no treatment modalities.[6] Clearly, there is an urgent need for the development of new antimicrobial agents that offer effective treatment against infections caused by resistant gram-positive bacteria.

A number of reports have appeared in the last five years describing new β-lactam agents with improved activity against MRSA. These compounds include cephems,[7] carbacephems,[8] oxaisocephems,[9] thiaisocephems,[10] penems,[11] and carbapenems.[12] A decade-long search for anti-MRSA compounds in our laboratories has led to a series of 2-aryl[13] and 2-benzothiazolylthio[14] carbapenems from which the highly evolved candidates L-695,256 (**1**),[15] L-742,728 (**2**)[16] and L-763,863 (**3**) were selected for extended evaluation. Each of these compounds contains a lipophilic side-chain which is further substituted with a cationic group. The lipophilic component provides for potent binding to the target penicillin binding proteins (PBPs), including the normally low-affinity PBP2a[17] (or PBP2') of MRS and the overproduced, low-affinity PBP5[18] of resistant enterococci.

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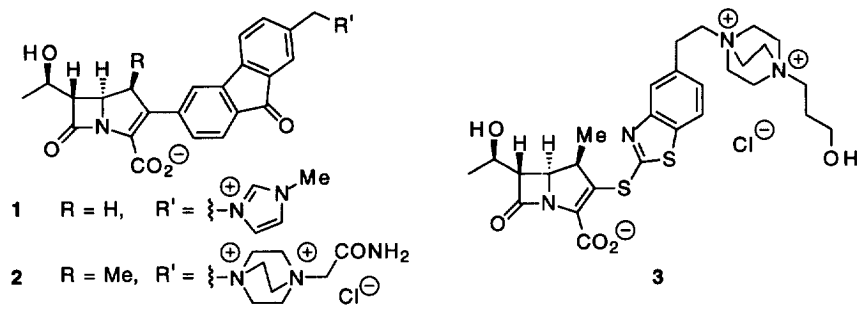
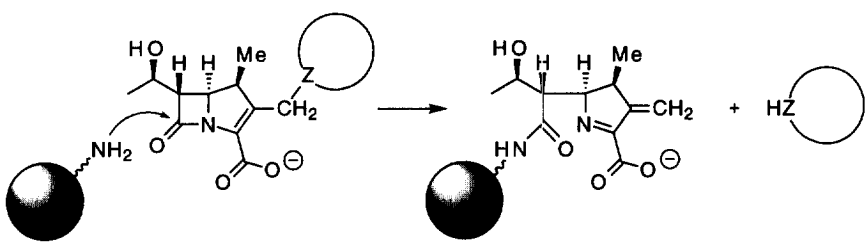
PII: S0960-894X(99)00070-0



While the carbapenems **1–3** were found to be safe and efficacious in a number of non-primate animal studies, they all exhibited signs of an immune-based toxicity[19] in a four-week safety study in rhesus monkeys. The immunotoxicity was believed to result from nonspecific acylation of proteinyl lysine residues by the carbapenem, followed by immune recognition and response to the appended hapten. Further, it was the lipophilic side-chain, essential for PBP2a binding and anti-MRSA activity, which was suspected of being the principle immunogenic component of the hapten. This paradox was addressed with the development of the "releasable hapten" hypothesis (see Figure 1). In its simplest form, we proposed to link the PBP2a binding, anti-MRSA pharmacophore to the 2-position of the carbapenem nucleus via a methylene spacer. By analogy to known cephalosporin[20] and penem[21] chemistry, opening of the β-lactam ring should result in expulsion of the immunogenic side-chain, thereby rendering it harmless.

Figure 1. The "Releasable Hapten" Hypothesis



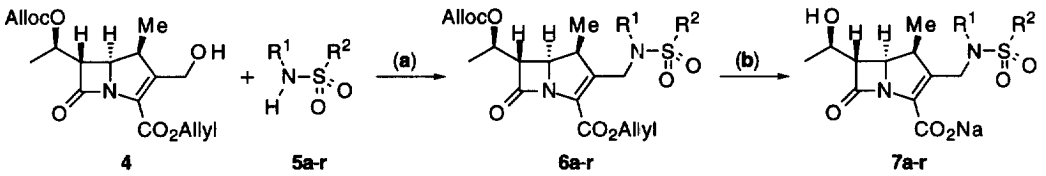
A variety of 2-substituted-methyl carbapenems bearing releasable, acidic groups are possible. This includes methyl-substituted ethers, thioethers, esters, carbamates and sulfonamides bearing lipophilic aromatic or heteroaromatic groups. In this paper, we describe our search for and identification of a 2-(sulfonamido)methyl carbapenem with potent activity against resistant gram-positive bacteria.

Chemistry

Aryl sulfonamides were targeted as potential anti-MRS pharmacophores for several reasons. The acidity of aryl sulfonamides[22] suggested that introduction of the side chain could easily be accomplished by Mitsunobu methodology[23] and, once introduced, the side chain would be readily expulsed upon β-lactam ring opening. Furthermore, substitution of the nitrogen or sulfur atoms would allow for the synthesis of a variety of acyclic and cyclic lipophilic derivatives (see Scheme 1). As anticipated, Mitsunobu reaction of sulfonamides **5a–r**[24] with the bis(allyl)-protected hydroxymethyl carbapenem **4**[25] proceeded without difficulty. The average yield for this step

was 49% with yields varying between 28% for **6j** and 76% for **6h**. Deprotection of intermediates **6a–r** using the McCombie protocol[26] produced analogs **7a–r**. The yields for the deprotection averaged 67% and ranged from 21% for **7p** to 100% for **7b**. The structures of representative side chains and the antibacterial activities of the final products against the homotypic MRSA strain COL are shown in Table 1.

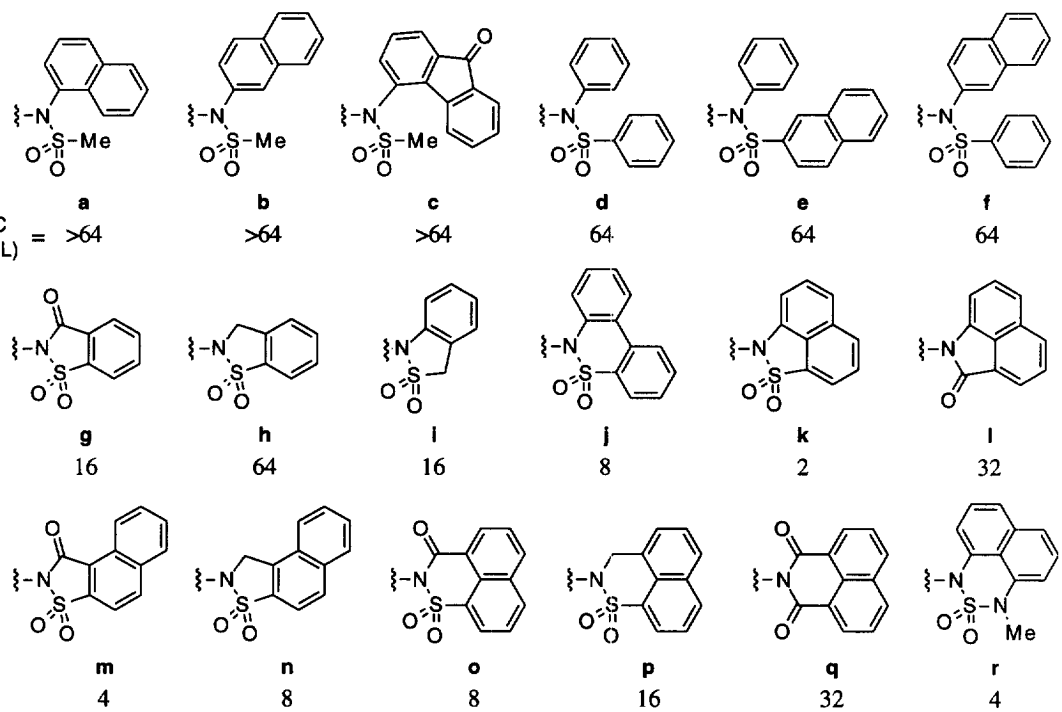
Scheme 1



4 5a-r 6a-r 7a-r

(a) DEAD or DIAD, PPh_3, THF, 0 °C; (b) $Pd(PPh_3)_4$, PPh_3, Bu-CHEt-CO_2Na, Bu-CHEt-CO_2H, CH_2Cl_2/EtOAc, 0 °C.

Table 1. Side Chain Structures[a] (-NR^1-SO_2R^2) and In Vitro Activity[b] against the Homotypic MRSA Strain COL for the (Sulfonamido)methyl Carbapenems **7a–r**



	a	b	c	d	e	f
MIC (μg/mL) =	>64	>64	>64	64	64	64

	g	h	i	j	k	l
	16	64	16	8	2	32

	m	n	o	p	q	r
	4	8	8	16	32	4

[a] Analogs **7l** and **7q** were prepared according to Scheme 1 by substituting the appropriate lactam for the sulfonamide **5**.

[b] Minimum inhibitory concentrations (MICs) for MRSA strain COL were determined by broth microdilution with Mueller-Hinton broth containing 2% NaCl. The MIC is defined as the lowest concentration of test compound that resulted in no visible growth after incubation at 35 °C for 22 h.

Results and Discussion

The following conclusions can be drawn from the data presented in Table 1, in which the homotypic MRSA strain COL was used to define the fundamental SAR. The acyclic sulfonamides **7a–f** are essentially inactive, regardless of the number or location of the aryl groups. The isomeric bicyclic sulfonamides **7h** and **7i** are poorly to moderately active; whereas, the more lipophilic tricyclic sulfonamides **7j**, **7k**, **7m**, **7n**, **7o**, and **7p** range from moderately active (**7p**) to highly active (**7k**). Interestingly, oxo substitution adjacent to the nitrogen atom in analogs **7h**, **7n**, and **7p** leads to the acylsulfonamides **7g**, **7m**, and **7o** that are two- to fourfold more active. The best of the tricyclic compounds, the 1,8-naphthosultamyl derivative **7k**, is two- to fourfold more active than its structurally closest relatives. For example, insertion of a carbonyl group (**7o**), a methylene group (**7p**), or an aza group (**7r**) into the sultam ring of **7k** leads to diminished activity, and replacement of the sulfonyl group by a carbonyl group (**7l**) dramatically reduces potency. The negative impact of sulfonyl group replacement is also apparent from the **7o**, **7q** pairing and from a comparison of saccharin derivative **7g** to the corresponding phthalimide (MIC 64 μg/mL, structure not shown).

Table 2. Antibacterial Activity[a] and Soluble PBP2a Binding[b] Data for Tricyclic (Sulfonamido)methyl Carbapenems **7k–r**

Compound	MIC values (μg/mL)					PBP2a IC_{50} (μg/mL)
	MRSA (12)	MRCNS (5)	VREFs (1)	MDREFm (3)	PRSP (2)	
7k	0.7	3.5	8	25.4	0.25	8.8
7l	12.0	21.1	64	64	4	--
7m	1.3	5.3	8	20.2	0.25	1.6
7n	3.4	10.6	8	50.8	0.17	17.7
7o	4.0	8.0	8	32	0.25	2.4
7p	8.5	16.0	8	40.3	0.12	21.8
7q	12.0	24.3	64	64	1.4	75.2
7r	1.5	7.0	64	64	1.0	7.1
Imipenem	>40	>64	8	>64	0.25	188
Vancomycin	0.9	1.7	>64	20.2	0.35	--

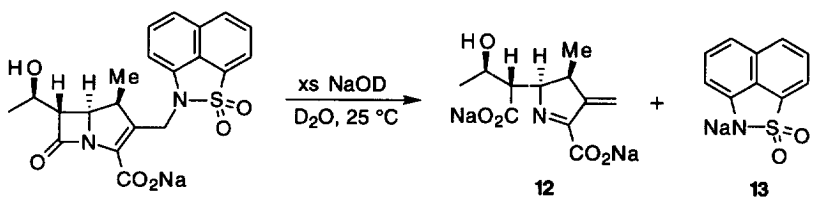
[a]MICs for all MRS strains were determined by broth microdilution with Mueller-Hinton (MH) broth containing 2% NaCl. MICs for enterococci were determined in brain heart infusion (BHI) broth and MICs for streptococci in either BHI or in MH broth supplemented with lysed horse blood. The activities are expressed as the geometric mean of the 20–22 h MICs for the number of strains indicated in parentheses. MRSA includes the homotypic strain COL, two homotypic/heterotypic strains, and nine heterotypic strains; MRCNS includes two heterotypic *Staphylococcus epidermidis*, one heterotypic *Staphylococcus hominis*, and two homotypic *Staphylococcus haemolyticus* strains; VREFs is a $Van^{R}Gent^{R}Ipm^{BR}$ *Enterococcus faecalis* strain; MDREFm includes one $Van^{S}Amp^{R}$ and two $Van^{R}Amp^{R}$ *Enterococcus faecium* strains; and PRSP includes a $Pen^{R}Tet^{R}Cam^{R}$ and a $Pen^{R}Tet^{R}Rif^{R}$ *Streptococcus pneumoniae* strain.

[b]Soluble PBP2a (0.6 μM) in 200 mM HEPES, pH 7, 150 mM NaCl was incubated with unlabeled carbapenem for 60 min at 30 °C followed by 60 min incubation with [^{3}H]-benzylpenicillin (50 μM). Protein was precipitated with 10% TCA, collected on vacuum filter plates, and radiolabelled protein complexes were quantitated. PBP2a binding is expressed as the concentration of test compound that inhibited the binding of [^{3}H]-benzylpenicillin by 50%.

A more detailed assessment of the antibacterial activities of the carbapenems **7k–7r** bearing tricyclic side-chains is presented in Table 2. The activities against the MRSA and MRCNS panels parallels the relative activities discussed above for the COL strain. This trend is also reflected in the binding affinities to a soluble form of PBP2a.[27] The compounds with the highest anti-MRS activity (**7k**, **7m**, **7o**, and **7r**) display the lowest IC_{50} values and, conversely, the least potent compounds, such as **7p**, **7q** and imipenem, display the highest IC_{50} values. The 1,8-naphthosultam carbapenem **7k** is the most active analog tested, with approximately twice the anti-MRS potency of its closest rivals **7m** and **7r**. The antibacterial activity of **7k** against the enterococcal and PRSP strains is similar to **7m**, but superior to analog **7r**. In addition, analog **7k** compares favorably with vancomycin, especially against the VRE strains.

Finally, it was necessary to demonstrate a fragmentation pattern consistent with the releasable hapten hypothesis. This was accomplished by examining the chemical degradation of the (naphthosultamyl)methyl analog **7k** by ^1H NMR (see Scheme 2). Addition of excess NaOD to a solution of **7k** in D_2O resulted in extremely rapid and quantitative ring-opening of the β-lactam accompanied by simultaneous appearance of the expulsed side-chain. The only observed products were the Δ^1-pyrroline **12** and the sodium salt of the 1,8-naphthosultam **13**. A ring-opened, non-fragmented intermediate was not observed in this experiment. These results are in complete accord with the releasable hapten hypothesis.

Scheme 2



7k **12** **13**

Summary

Based on a desirable in vitro activity profile and an appropriate chemical fragmentation pattern, the 1,8-naphthosultam platform was selected for further derivitization studies with the goal of developing a clinically useful, anti-MRS carbapenem antibiotic. Several of the key issues that remained to be addressed included reducing serum protein binding, improving pharmacokinetic parameters, and increasing potency against the target pathogens. These studies are the subject of a second communication from these laboratories.[28]

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