Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 11, pp. 1409-1414, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00230-8

STUDIES ON β-LACTAM ANTIBIOTICS. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL 1β-METHYLCARBAPENEMS RELATED TO FR21818: 5-MEMBERED RING ANALOGS

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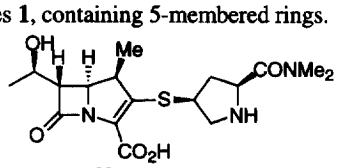
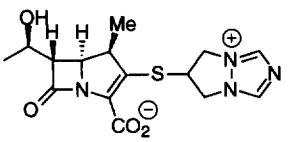
Abstract: The synthesis and biological activity of the novel series of 1β-methylcarbapenems **1** are described. Most compounds displayed extremely potent antibacterial activity and high renal DHP-I stability. The best compound in this series, FR21818 (**1a**) displayed excellent *in vivo* efficacy against an MRSA infection in mice.

Introduction

Increasing incidence of bacterial strains resistant to available antibiotics requires the discovery of new agents with more potent activity. In the continuing search for new carbapenems[1] with superior antibacterial activity compared to agents such as imipenem,[2] meropenem, and biapenem, we earlier postulated that a combination of a high affinity for penicillin-binding proteins with high bacterial membrane permeability would lead to a superior profile and may be achieved by incorporation of quaternary salts of heterocycles onto a pyrrolidine ring in novel C2-substituents.[3,4] As a result of these efforts we discovered FR21818 (**1a**)[3] and FR21751 (**1b**)[4] which both possessed excellent, broad spectrum activity against Gram-positive and Gram-negative bacteria and good stability to renal dehydropeptidase-I (DHP-I). Furthermore, whilst **1b** displayed an unusual tendency to epimerize at the pyrrolidine 2-position in aqueous solution (>pH 6),[4] FR21818 was completely stable to epimerization under similar conditions. In this paper we thus explore in greater detail the structure activity relationships of azoles related to FR21818, and in particular the synthesis, antibacterial activity, and stability to DHP-I of the series **1**, containing 5-membered rings.



Meropenem



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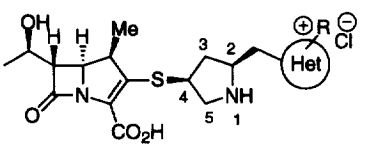

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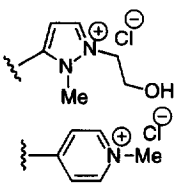


FR21818 (1a)

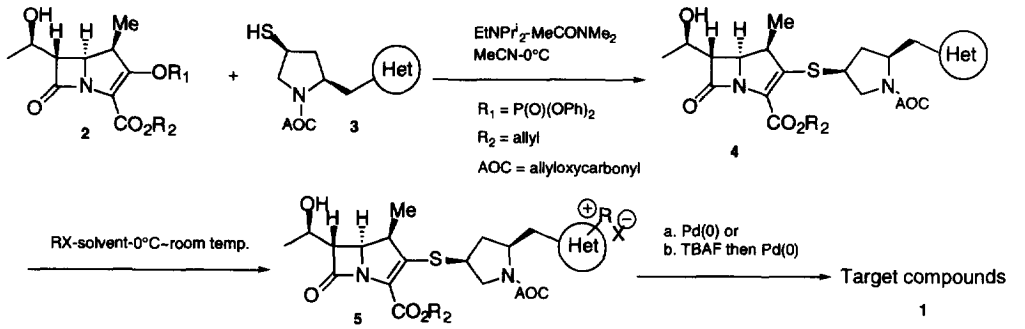
FR21751 (1b)

Synthesis

New carbapenems described in this paper were prepared by adaptation of the methods used for FR21818 (**1a**) and FR21751 (**1b**), as described in our earlier reports,[3,4] and are summarized in Schemes 1 and 2. The appropriate thiols **3** were coupled with activated carbapenem **2** leading to protected carbapenems **4**, converted to

quaternary salts **5** with an alkyl halide or triflate, and deprotected under Pd-catalyzed conditions to afford the final compounds **1** as amorphous solids after lypholization. In the case of compounds **1g~h** and **1j~k**, a different approach was necessitated by the low reactivity of the available alkylating agents, and involved formation of the pyrazolium salt of **3** before coupling with the carbapenem **2**.[5] Bicyclopyrazole compound **1v** was obtained from the appropriate acyclic hydroxypropyl pyrazole carbapenem precursor **4** by treatment with triflic anhydride; triflate formation was spontaneously followed by intramolecular cyclization; and the usual Pd-catalyzed deprotection step. Activated carbapenem **2** was obtained as indicated previously,[3] and the thiol components were mostly prepared by application, with modification where required, of our published route to the pyrazole intermediate for FR21818 (**1a**) (Scheme 2).[3]

Scheme 1. General Synthetic Route to Novel Carbapenems (**1**)



Scheme 2. Synthesis of Key Thiol Precursors (**13**)

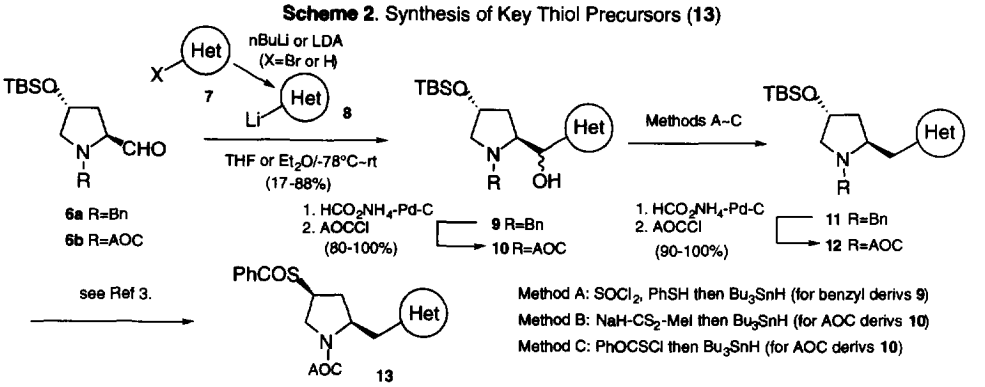
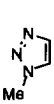
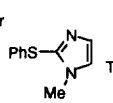
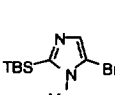
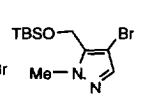
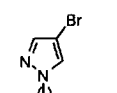


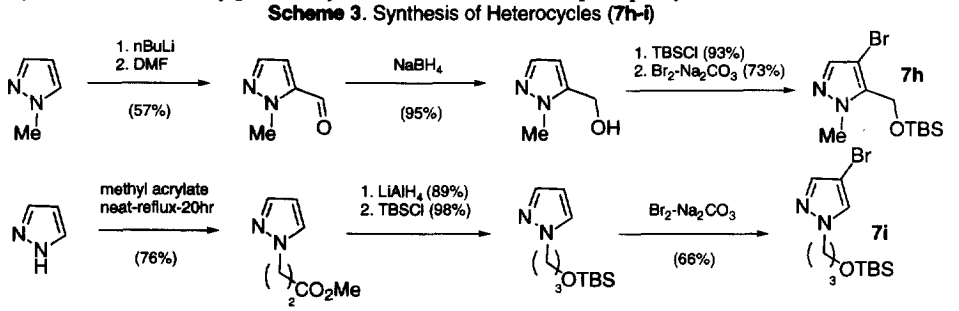
Table 1. Heterocycles **7**; Yields of **9**; Deoxygenation Method and Yield of **11** or **12**

Heterocycle									
	7a	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**
Yield of **9**	60%	88%	75%	48%	65%	56%	47%	44%	17%(**10**)
Deoxygenation Method	A	B	C	B	C	A	A	B	B [from **6b**]
Yield	47%	82%	85%	100%	57%	66%	66%	57%	29%

Thiols **3** were prepared from the thiobenzoates **13**, which were obtained by the general route summarized in Scheme 2. Addition of the lithium anions **8**, derived from heterocycles **7a~i** by deprotonation or lithium-bromine exchange, to aldehyde **6a** gave diastereomeric mixtures of alcohol adducts **9**, except in the case of **7i**, which was coupled with **6b** to give the corresponding **10**. Deoxygenation to afford intermediates **11** or **12** was performed by one of several methods. Tin hydride-mediated reduction of a xanthate, thiocarbonate, or phenylthio ether derivative gave key intermediates **11** or **12** in high yield. In the cases involving use of heterocycles **7f~i**, additional de-blocking-blocking steps were required in order to derive intermediates **13**.[6] Intermediates for substituted azoles **1q~r** and **1t** were obtained by functionalization of the corresponding N-benzyl azoles **11**. Lithium anion formation (directly in the case of the imidazole; after bromination in the case of the pyrazole) was followed by quenching with a suitable one-carbon unit (DMF or $ClCO_2Me$). Further manipulations gave the requisite intermediates **13** in good yield. The only exception to this general route was that used to prepare the intermediates for **1s**.[7]

Heterocycles **7a~b** were commercially available. **7c**,[8a] **7d**,[8b] **7e**,[8c] and **7f**[8d] were prepared by standard literature methods. **7g** was obtained by lithiation and silylation of N-methylimidazole; the resulting product was then regioselectively brominated (NBS). More highly functionalized pyrazoles **7h** and **7i** were prepared by the sequence of steps outlined in Scheme 3. Thus, regioselective formylation of N-methylpyrazole (nBuLi-THF, DMF), followed by sodium borohydride reduction of the resulting aldehyde, silylation and regioselective bromination at C4 gave **7h** in good yield. **7i** was obtained in 4 steps by conjugate addition reaction of pyrazole with methyl acrylate, methyl ester reduction ($LiAlH_4$), silylation, and finally regioselective bromination. **7i** could also be alternatively obtained directly from commercially available 4-bromopyrazole and the monosilyl ether of propane-1,3-diol by a straightforward Mitsunobu reaction (Ph_3P-DEAD-THF-room temp-68%). Heterocycles **7** were routinely purified by distillation to ensure adequate purity for the metallation reactions.

Scheme 3. Synthesis of Heterocycles (**7h-i**)



Biological Activity

(a) *In Vitro* Antibacterial Activity and DHP-I Stability

In vitro antibacterial activity and DHP-I stability of the new carbapenems prepared in this work are shown in Table 2. Standard serial dilution techniques were used for MIC determinations. The recombinant human enzyme was used for measurement of DHP-I stability, which is represented as the relative rate of hydrolysis compared to meropenem (rate=1.0). Both meropenem and biapenem are included as reference drugs. Our first goal was to determine which, if any, unsubstituted heterocyclic system gave the best spectrum of activity and stability when the alkyl group R was a simple methyl substituent, in order to narrow the possible range of derivatives for further variation. Comparison of the regioisomeric imidazoles **1c** and **1o**, pyrazoles **1f** and **1i**, and triazoles **1m** and **1p** indicates that whilst activity against bacteria such as *Escherichia coli* NIHJ JC-2 is not greatly variable, quite wide differences are apparent with regards susceptibility of clinically significant species such as

Staphylococcus aureus 3004 (a strain of MRSA) and *Pseudomonas aeruginosa* IAM 1095. Thus, whilst **1c** displays MIC's of 6.25 and 3.13 μg/ml against these two strains, the isomeric imidazole **1o** is significantly weaker; 6.25 and 12.5 μg/ml respectively. Of the pyrazole isomers **1f** and **1i**, the most potent activity against *Staphylococci* was displayed by **1i**, whilst the most potent activity against *Pseudomonas* was displayed by **1f**. Indeed, the MIC value of **1f** against *P.aeruginosa* IAM 1095 (1.56 μg/ml) was amongst the most potent of all derivatives prepared, and equal to the reference drugs meropenem and biapenem. Triazoles **1m** and **1p** displayed the same trends as with the imidazole compounds. The weakest analog **1p** possessing the two alkyl substituents in a flanking arrangement about the point of attachment to the pyrrolidinemethyl group, similar to **1o**. Whilst the source of the lowering of activity is unclear, it is tempting to suggest a change in conformation about the pyrrolidine ring as a result of the extreme steric congestion present in **1o** and **1p**. From these initial results, it became apparent that we should focus our attention on 4- and 5-pyrazoles and 5-imidazoles. 1,2,3-triazoles were eliminated due to the weak activity of **1m** against the MRSA strain *S.aureus* 3004 (25 μg/ml), activity not improved in the hydroxyethyl analog **1n**.

Changing the alkyl substituent R from methyl to polar substituents, such as hydroxyethyl, carboxymethyl, and carbamoylmethyl, was examined with the hope of improving activity in comparison to the parent heterocycle. This was expected to result from an electron-withdrawing effect and a decrease in the electron density at the quaternary ammonium center. As shown in Table 2, carbamoylmethyl imidazole **1d** and hydroxymethyl imidazole **1e** had slightly improved stability to DHP-I, but slightly weaker activity against MRSA. The same trend was apparent with the 4-pyrazole series **1j~l**, the only significant result being the improved activity of **1l** against *P.aeruginosa* IAM 1095. In the 5-pyrazole series, the potent activity of the parent **1f** against resistant *Staphylococci* was retained in the analogs **1g** and **1a**. Activity against typical Gram-negative bacteria was reduced only slightly. Overall, 5-pyrazoles gave the best balance of activity versus both types of significant bacteria, although major improvements in antibacterial activity over parent heterocycles was rare.

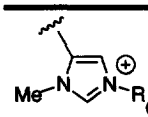
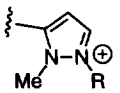
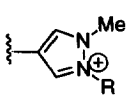
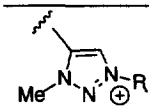
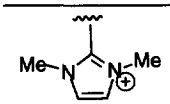
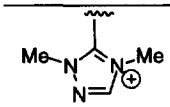
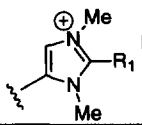
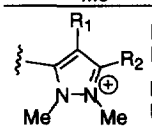
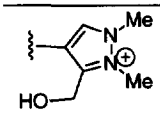
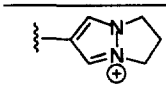
We examined the effect of additional substituents on the heterocyclic ring of the most potent series. Carbapenems **1q** and **1r**, containing hydroxymethyl and carbamoyl groups respectively, were equipotent with the parent imidazole **1c** against most strains, including *S.aureus* 3004. The only exception was *P.aeruginosa* IAM 1095; both **1q** and **1r** displayed more potent activity against this strain than the parent **1c**. Hydroxymethyl-substituted pyrazoles **1s** and **1t** were less active than the parent pyrazole **1f**, indicating a variable effect. The lower activity of **1s** confirms further the incompatibility of flanking substituents. Pyrazole **1u** and bicyclic pyrazolium **1v** displayed slightly lower activity compared to the parent **1i**, and were not pursued further.

Amongst these compounds, the most promising candidates, in particular the 5-pyrazoles, were further examined for acute toxicity upon i.v. administration in rats. As a result, FR21818 (**1a**) displayed the best profile. Furthermore, since physicochemical properties are very important for subsequent development, the ready crystallization of FR21818 from ethanol-water, in sharp contrast to several other analogs, provided the compound with the best overall profile for further pharmacokinetic and efficacy investigation.

(b) *In Vivo* Protective Activity of FR21818

The *in vivo* protective activity of FR21818 against lethal systemic infection by *S.aureus* 8008 (MRSA) in ICR mice was investigated. Compound was administered subcutaneously one hour after intraperitonial infection with 1-5 times the minimum lethal dose. Activity was calculated as the 50% effective dose; as a result, FR21818 displayed an ED_{50} of 5.45 mg/kg, in comparison with 2.73 mg/kg for vancomycin, 7.02 mg/kg for imipenem/cilastatin, 7.88 mg/kg for meropenem/cilastatin, and 35.2 mg/kg for biapenem. This result indicates that FR21818 is a very potent new carbapenem with a good potential for treatment of MRSA infections.

Table 2. *In vitro* Antibacterial Activity and DHP-I Stability of Novel Azoliomethyl Carbapenems (**1**)

Het⊕ R	R	MIC (μg/ml)						DHP-I Stability
		S.a.1	*S.a.2*	*E.c.*	*P.v.*	*P.a.1*	*P.a.2*	
	Me (**1c**)	0.05	6.25	0.20	0.78	0.20	3.13	0.41
	CH_2CONH_2 (**1d**)	0.05	12.5	0.10	0.78	0.20	3.13	0.31
	CH_2CH_2OH (**1e**)	0.05	12.5	0.10	1.56	0.39	3.13	0.25
	Me (**1f**)	0.05	6.25	0.20	0.78	0.20	1.56	0.42
	CH_2CONH_2 (**1g**)	<0.025	6.25	0.10	0.39	0.10	3.13	0.35
	CH_2CO_2H (**1h**)	n.d.	n.d.	0.05	n.d.	n.d.	3.13	0.61
	CH_2CH_2OH (**1a**)	0.05	6.25	0.20	1.56	0.20	6.25	0.26
	Me (**1i**)	0.05	3.13	0.20	1.56	0.39	3.13	0.28
	CH_2CONH_2 (**1j**)	<0.025	6.25	0.20	0.78	0.20	3.13	0.29
	CH_2CO_2H (**1k**)	0.10	12.5	0.10	0.78	0.39	6.25	0.14
	CH_2CH_2OH (**1l**)	0.05	12.5	0.39	1.56	0.39	1.56	0.20
	Me (**1m**)	0.05	25	0.10	0.78	0.39	3.13	0.32
	CH_2CH_2OH (**1n**)	0.10	25	0.10	0.78	0.39	3.13	0.17
	(**1o**)	0.10	6.25	0.10	0.78	0.39	12.5	0.30
	(**1p**)	0.10	12.5	0.20	0.78	1.56	12.5	0.22
	R_1=CH_2OH (**1q**)	0.05	6.25	0.20	0.78	0.20	1.56	0.27
	R_1=$CONH_2$ (**1r**)	n.d.	n.d.	0.10	n.d.	n.d.	1.56	0.30
	R_1=H, R_2=CH_2OH (**1s**)	n.d.	n.d.	0.10	n.d.	n.d.	6.25	n.d.
	R_1=CH_2OH, R_2=H (**1t**)	0.10	25	0.20	0.78	0.39	3.13	0.28
	(**1u**)	0.05	6.25	0.39	1.56	0.39	6.25	0.24
	(**1v**)	n.d.	n.d.	0.10	n.d.	n.d.	3.13	0.21
Meropenem		0.10	25	0.025	0.10	0.20	1.56	1.0
Biapenem		0.05	25	0.39	3.13	0.20	1.56	0.15

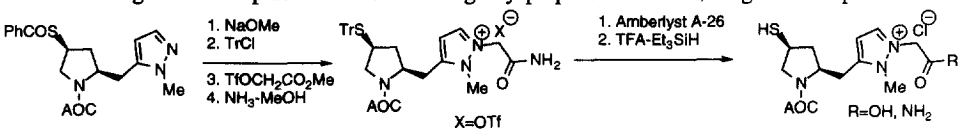
S.a.1=*S.aureus* 209P JC-1; *S.a.2*=*S.aureus* 3004(MRSA); *E.c.* =*E.coli* NIHJ JC-2; *P.v.*=*P.vulgaris* IAM 1025; *P.a.1*=*P.aeruginosa* 26; *P.a.2*=*P.aeruginosa* IAM 1095. n.d. = not determined

Conclusions

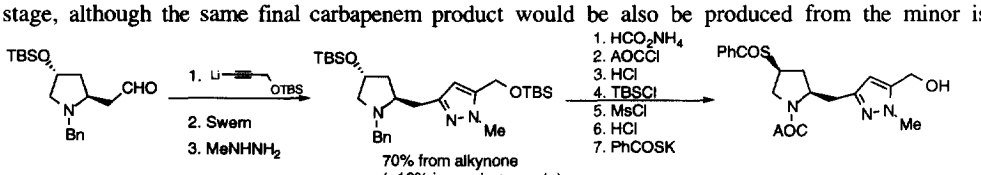
In this paper, we have reported the synthesis and *in vitro* antibacterial activity of 1β-methylcarbapenems containing novel azoliomethyl substituted pyrrolidine substituents related to our previously described FR21818 (**1a**). Most of the prepared compounds displayed potent, broad spectrum antibacterial activity and high stability to renal DHP-I. From these compounds, FR21818 was selected for further pre-clinical evaluation on the basis of overall balance of activity, *in vivo* efficacy, physicochemical properties, and preliminary toxicological evaluation. Future publications from these laboratories will disclose these results in greater detail.

References and Notes

1. For a recent review on the chemistry and biology of carbapenems, see: Coulton, S.; Hunt, E. in *Progress in Medicinal Chemistry*, Vol. 33, pp 99-145 (1996); Eds. Ellis, G.P.; Luscombe, D.K., Elsevier.
2. Clinical experience with the Imipenem-Cilastatin combination was reviewed recently, see: Buckley, M.M.; Brogden, R.N.; Barradell, L.B.; Goa, K.L. *Drugs* **1992**, *44*, 408.
3. Azami, H.; Barrett, D.; Tanaka, A.; Sasaki, H.; Matsuda, K.; Chiba, T.; Matsumoto, Y.; Matsumoto, S.; Morinaga, C.; Ishiguro, K.; Tawara, S.; Sakane, K.; Takasugi, H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2199.
4. Azami, H.; Barrett, D.; Chiba, T.; Fujikawa, A.; Sakane, K.; Shirai, F. *Chem. Pharm. Bull.* **1997**, *45*, 209.
5. We developed an alternative synthesis of the thiols required for **1g** and **1j**, illustrated below for the 5-pyrazole series, employing quaternary salt formation prior to carbapenem coupling. Coupling in the standard way, followed by Pd-catalyzed deprotection afforded the final compounds. Trace amounts (<10%) of the carboxylic acid derivatives, arising from competing ester hydrolysis, were carried through to the final antibacterial agents and separated at the final stage by preparative HPLC, to give carbapenems **1h** and **1k**.



6. Coupling of **6a** with **7f** (LDA-THF) was followed by desulfurization with Raney Ni in ethanol, to give the corresponding alcohol **9**.[8d] The same compound was more conveniently obtained on a large scale by coupling of **6a** with the anion derived from **7g**, and treatment of the crude worked-up product with AcOH-MeOH to effect desilylation.
7. The key thiobenzoate intermediate for carbapenem **1s** was not obtained by application of the general method. Instead, the pyrazole ring was constructed by the multi-step sequence outlined below, involving condensation of methylhydrazine with an alkynone intermediate obtained from an aldehyde. A regioisomeric mixture of pyrazoles was obtained from this process, with the separated major isomer being carried forward to the final stage, although the same final carbapenem product would be also be produced from the minor isomer.



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(*Received in Japan* 17 *March* 1997; *accepted* 25 *April* 1997)