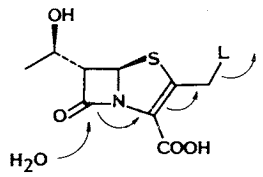
DUAL-ACTION PENEMS

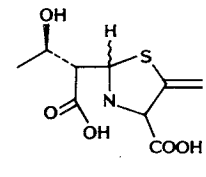
Sir:

Great attention is being devoted to quinolylcephalosporins[1~4], bifunctional antibiotics devised as an extension of the therapeutic principle of incorporating a latent antibacterial agent at the C-3′ position of the cephem ring[5]. Bifunctional compounds within the penem class were originally conceived by us after the theoretical prediction[6,7] and experimental observation[6,8] that β-lactam cleavage of penems (**1**) carrying leaving groups at the C-2′ position results in the release of such groups (**2**) and of a major lactam-opened metabolite (**3**). The synthesis of the first penem with a putative dual mode of action, the imide-linked nitrofurantoyl derivative **1a**, was anticipated some years ago[9]. We have pursued this concept further[10] with ether-linked (**1b**), carbamate-linked (**1c**, **1d**) and ester-linked (**1e**~**1i**) compounds. Here we wish to communicate preliminary results of our research program, which includes the new ex-

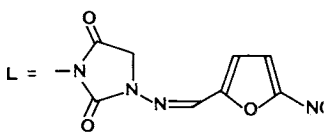


1a~**1i**

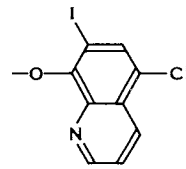
2a~**2i**



3 (*R*-epimer)
4 (*S*-epimer)



a



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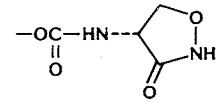

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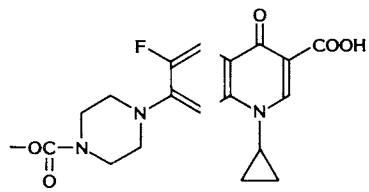

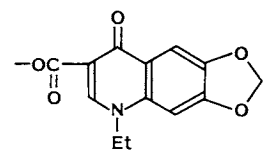

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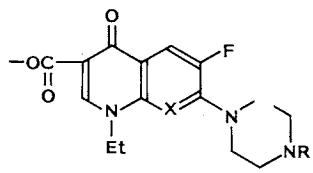
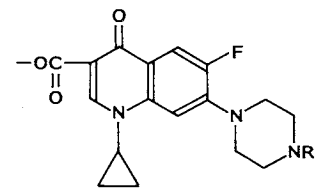
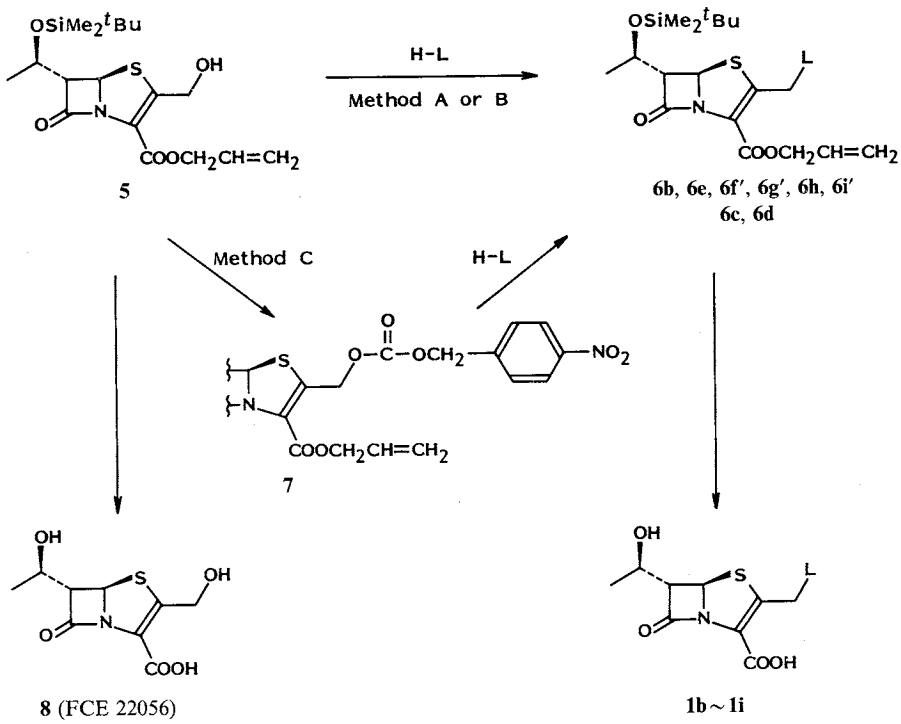


f X=N R=H
g X=CH R=H
h X=CH R=$COOC_2H_5$
f′ X=N R=$CO_2CH_2CH{=}CH_2$
g′ X=CH R=$CO_2CH_2CH{=}CH_2$



i R=H **i′** R=$CO_2CH_2CH{=}CH_2$



citing class of quinolyl-penems (**1d**~**1i**).

All compounds were prepared by using the 2-hydroxymethylpenem derivative **5**[11] as a template on which the second antibacterial agent could be accommodated. Three different condensation procedures were used.

According to Method A (MITSUNOBU[12] condensation), the carbinol **5** was coupled with iodochlorhydroxyquin (**2b**) in the presence of a slight molar excess of PPh_3-diethyl azodicarboxylate complex (THF, 30 minutes) to afford **6b** in 70% yield. Activation of the carboxyl group of quinolones as the mixed ethylcarbonic anhydride was preferred for preparing the ester-linked quinolyl-penems (Method B). Typically, oxolinic acid (**2e**) was treated with ethyl chlorocarbonate (NEt_3, CH_2Cl_2-DMF, 0°C) and then with **5** (4 hours, 25°C) to provide **6e** (56%). The piperazine-substituted quinolones enoxacin (**2f**), norfloxacin (**2g**) and ciprofloxacin (**2i**) required protection of the terminal nitrogen atom. Acylation with allyl chlorocarbonate under Schotten-Baumann conditions (0.2 N NaOH-acetone 10:1) gave **2f'**, **2g'**, **2i'**, which were condensed with **5** to afford **6f'**, **6g'** and **6i'**. By substituting ethyl chlorocarbonate for allyl chlorocarbonate, the norfloxacin derivative **2h** was analogously prepared and converted to **6h**. Finally, the carbamate-linked compounds **1c** and **1d** were addressed (Method C) by converting **5** into the mixed carbonate **7** (*p*-nitrobenzyl chlorocarbonate, NEt_3, THF, 3 hours; 85% yield). Selective expulsion of *p*-nitrobenzyl alcohol occurred upon condensation with D-cycloserine (DMF, 4 hours) and with the sodium salt of ciprofloxacin (DMF, overnight) to afford **6c** (70%) and **6d** (90%), respectively. Deblocking of obtained compounds **6b**~**6e**, **6f'**, **6g'**, **6h**, **6i'** was achieved by procedures popular in penem chemistry, *i.e.* desilylation (Bu_4NF $3H_2O$, HOAc-THF, overnight) and palladium-mediated transallylation with sodium 2-ethyl-hexanoate ($Pd(PPh_3)_4$ 0.1 mol equiv, PPh_3, THF-CH_2Cl_2 1:1, 1 hour). The final products were isolated as sodium salts (**1b**~**1e**, **1h**) or zwitterions (**1f**, **1g**, **1i**) after repeated purification by reverse-phase chromatography on C_{18}-silica.

The *in vitro* antibacterial activity of dual-action penems **1** is reported in Table 1 together with the activity of the latent antibacterial agents **2** incorporated in the penem molecules; the reference penem FCE 22101[13] and the carrier penem FCE 22056[14] (**8**) are also included. All of the investigated bifunctional penems, except the cycloserine derivative **1c**, showed very good activity on Staphylococci and Streptococci, while the activity on Gram-negative organisms seemed to reflect more closely the contribution of the second component, varying from very poor (**1h**) to excellent (**1d**, **1i**). A more

Table 1. *In vitro* antibacterial[a,b] activity of penems **1a**~**1i**, carried molecules **2a**~**2i**[c] and reference compounds.

	S.a.	*S.a.* P+	*S.a.* Q+	*S.a.* MR	*S.p.*	*S.f.*	*E.c.*	*E.c.* DC2	*E.c.* +	*K.a.* +	*E.cl.* +	*S.m.*	*S.m.* +	*Pr.m.*	*P.a.*	*B.f.*	*Pe.m.*	*C.p.*	*C.d.*
1a	0.09	0.78	nd	12.5	0.04	6.25	3.12	0.39	3.12	1.56	12.5	6.25	>50	12.5	>50	nd	nd	nd	nd
2a	12.5	25	nd	12.5	12.5	50	12.5	6.25	12.5	12.5	12.5	>50	>50	>50	>50	nd	nd	nd	nd
1b	0.005	0.01	nd	0.19	0.001	0.78	6.25	0.04	6.25	12.5	12.5	50	50	25	25	nd	nd	nd	nd
2b	12.5	12.5	nd	12.5	25	25	25	6.25	25	50	50	>50	>50	50	12.5	nd	nd	nd	nd
1c	0.78	1.56	12.5	12.5	1.56	>50	3.12	3.12	3.12	3.12	50	>50	>50	6.25	>50	nd	nd	nd	nd
2c	12.5	25	25	25	25	50	25	25	25	>50	50	>50	>50	>50	>50	nd	nd	nd	nd
1d	0.09	0.09	0.39	0.78	0.02	6.25	1.56	0.39	0.09	0.19	0.09	1.56	12.5	0.39	0.78	6.25	0.19	0.78	50
2d	0.09	0.78	>50	0.78	0.78	25	0.19	0.09	0.005	0.01	0.01	0.19	0.78	0.04	0.09	3.12	0.78	0.78	12.5
1e	0.04	0.04	0.39	0.78	0.05	3.12	6.25	0.19	1.56	1.56	0.78	1.56	12.5	0.78	25	3.12	0.04	0.09	3.12
2e	1.56	1.56	6.25	1.56	25	50	3.12	0.78	0.19	0.19	0.01	0.19	6.25	0.09	6.25	>50	>50	1.56	>50
1f	0.09	0.19	1.56	1.56	0.01	3.12	3.12	0.78	1.56	1.56	1.56	6.25	>50	3.12	6.25	0.19	0.09	0.39	1.56
2f	0.39	1.56	>50	1.56	6.25	12.5	1.56	1.56	0.09	0.19	0.09	0.19	3.12	0.39	0.78	25	12.5	12.5	>50
1g	0.04	0.09	0.78	0.78	0.01	1.56	0.78	0.39	0.19	0.39	0.19	3.12	25	0.39	1.56	3.12	0.04	0.19	3.12
2g	0.39	1.56	>50	0.39	1.56	6.25	0.39	0.19	0.04	0.09	0.04	0.39	3.12	0.09	0.39	25	0.78	0.39	50
1h	0.09	0.19	0.78	0.78	0.01	3.12	>50	3.12	50	>50	25	>50	>50	>50	>50	50	0.04	0.78	12.5
2h	1.56	1.56	>50	1.56	>50	>50	>50	25	25	>50	25	>50	>50	>50	>50	>50	12.5	>50	>50
1i	0.04	0.09	0.39	0.78	0.01	1.56	0.78	0.19	0.04	0.09	0.04	0.78	6.25	0.19	0.39	3.12	0.09	0.39	6.25
2i	0.09	0.78	>50	0.78	0.78	25	0.19	0.09	0.005	0.01	0.01	0.19	0.78	0.04	0.09	3.12	0.78	0.78	12.5
FCE 22101	0.04	0.09	0.39	0.39	0.04	3.12	0.78	0.78	0.78	0.78	1.56	6.25	12.5	1.56	50	0.39	0.09	0.39	6.25
FCE 22056	0.19	0.19	0.78	0.78	0.19	12.5	3.12	3.12	3.12	3.12	6.25	12.5	12.5	12.5	50	0.39	0.78	1.56	25

[a] MICs (μg/ml) were determined by the standard 2-fold agar dilution method in Müller-Hinton for aerobic strains and in Wilkins Chalgren for anaerobes using an inoculum of 10^4 colony forming units/plate.

[b] Organisms included in this table are: *S.a.*, *Staphylococcus aureus* ATCC 13709; *S.a.* P+, *Staphylococcus aureus* 39/2 (penicillinase producer); *S.a.* Q+, *Staphylococcus aureus* CDB (resistant to quinolones); *S.a.* MR, *Staphylococcus aureus* 2101 (resistant to methicillin); *S.p.*, *Streptococcus pyogenes* ATCC 12384; *S.f.*, *Streptococcus faecalis* ATCC 6057; *E.c.*, *Escherichia coli* UB1005; *E.c.* DC2, *Escherichia coli* DC2 (OM-defective mutant); *E.c.* +, *Escherichia coli* R6K TEM-1 (producer of plasmid mediated β-lactamase); *K.a.* +, *Klebsiella aerogenes* 1082E (producer of chromosomally mediated β-lactamase); *E.cl.* +, *Enterobacter cloacae* P99 (producer of chromosomally mediated β-lactamase); *S.m.*, *Serratia marcescens* ATCC 2902; *S.m.* +, *Serratia marcescens* F52 (producer of chromosomally mediated β-lactamase); *Pr.m.*, *Proteus mirabilis* FI7474; *P.a.*, *Pseudomonas aeruginosa* 1771; *B.f.*, *Bacteroides fragilis* ATCC 25285; *Pe.m.*, *Peptococcus magnus*; *C.p.*, *Clostridium perfringens* ATCC 13124; *C.d.*, *Clostridium difficile* CD1.

[c] Antibacterial agents incorporated in penems **1** are: **2a**, nitrofurantoin; **2b**, iodochlorhydroxyquin; **2c**, cycloserine; **2d**=**2i**, ciprofloxacin; **2e**, oxolinic acid; **2f**, enoxacin; **2g**, norfloxacin; **2h**, *N*-ethoxycarbonyl-norfloxacin derivative. For convenience, **2c** and **2d** are represented in the formulae as their carbamic acid derivatives, the way they are released from the carrier penems **1c,d**; decarboxylation to the free amines occurs instantaneously.

nd: Not determined.

detailed discussion of the observed activity requires separating the contribution from a) the intact molecule, b) the second antibacterial agent, which might be released either in the culture medium or inside the bacterial cell, and c) any other bioactive penem, in particular FCE 22056, which might arise from cleavage of the chemical bridge between the two components.

Model experiments were set up to solve, at least in part, these ambiguities. The chemical stability (0.05 M pH 7.4 phosphate buffer, 37°C) of the intact molecule was determined by HPLC; obtained half-life values ranged from 22 hours (**1a**) to 81 hours (**1d**). Since FCE 22056 is even more stable (89 hours), but was never found at any time in the degradation mixtures, selective cleavage of the link between the two components is unlikely to occur under *in vitro* conditions. None the less, release of the second antibacterial agent was observed in the foregoing experiments. This event was quantitated (HPLC) for compounds **1a**, **1b**, **1d** and **1i** under alkaline conditions (0.05 M NaOH, 25°C), which allow complete hydrolysis in short times and the simultaneous determination[8] of the opened β-lactam metabolite **3**, unstable in neutral or acidic media. Molar recoveries (% of theoretic value) were: nitrofurantoin from **1a**, $\leq$10%; iodochlorhydroxyquin from **1b**, 77%; ciprofloxacin from **1d**, 88%; ciprofloxacin from **1i**, 56%. The expected lactam-opened fragment **3** was accompanied by a minor proportion (*ca.* 1:7) of its 5*S*-epimer **4**[15]; collectively, the molar recovery of the two well correlated with that of the second component **2**: $\leq$10% from **1a**; 91% from **1b**; 73% from **1d**; 59% from **1i**. Thus, according to our original rationale, a latent antimicrobial agent linked at the C-2′ position of the penem molecule through an ester, carbamate or aryl ether bridge is efficiently expelled upon β-lactam cleavage of the carrier penem moiety. The poor performance of the imide link of **1a** in this respect was found to be due to preferential hydrolysis of the hydantoin ring over the β-lactam ring.

The results above confirm that bifunctional compounds of the penem class have the potentiality to behave as true dual-action antibacterial agents, *i.e.* to inhibit peptidoglycan synthesis ("penem-like" action) and in this process (or in a parallel process occurring inside the bacterial cell) allow site-specific delivery of a latent antibiotic endowed with a different mode of action (*e.g.*, inhibition of bacterial DNA gyrase). As pointed out before, a contribution from the C-2′ linked component to the *in vitro* antibacterial activity is indeed apparent (Table 1) for the selected group of penems carrying a fluoroquinolone (**1d**, **1f**, **1g**, **1i**); contribution from other carried moieties, if ever occurs, is concealed by the superior activity of the whole molecule. The question remains as to whether the expanded spectrum of quinolyl-penems is not the result of a mere association of two agents (a penem and a quinolone), each separately present in the culture medium. Extensive biochemical studies on quinolyl-cephalosporins, designed for determining the relative importance of the antibacterial activity due to the whole molecule versus that due to the separate components, left the problem largely unresolved[1,4]. None the less, we believe that questions concerning quinolyl-penems can get answers from our preliminary results.

Chemical stability studies revealed that the link between the two components of quinolyl-penems is much more stable than that of quinolyl-cephalosporins, which reportedly[1,4] have chemical half-lives of about 3~10 hours (esters and carbamates, respectively). Corresponding values for **1i** (65 hours) and **1d** (81 hours), being similar to that of FCE 22101 (85 hours) and other ordinary penems, suggest that hydrolysis occurs at the β-lactam first; in agreement, release of FCE 22056 was never observed. Thus, a) the quinolyl-penems are enough stable to fully exert their antimicrobial activity as such, and b) contribution from other penem species released from the original molecule, *e.g.* FCE 22056, can be safely excluded. Now, the *in vitro* data of Table 1 lend themselves to a less equivocal interpretation, crediting potent activity on Gram-positive bacteria to most of the bifunctional compounds as such. The first evidence is that in several cases the activity observed impressively surpasses that of the carried molecule. This "penem-like" mode of action of the intact molecule is substantiated by results of penicillin-binding proteins (PBPs) affinity studies (see below). An additional evidence is the activity of the products on anaerobic organisms, which is a distinct feature of penems. Further, quinolyl-penems display excellent activity on the quinolone-resistant strain of *Staphylococcus aureus* included in Table 1. Moreover, it was apparent that the penems characterized by an aryl ether bridge (**1b**) or an ester bridge (including the oxolinic acid derivative **1e**) are active *per se* on methicillin-resistant strains of *S. aureus* (MRSA), in accordance with results of a parallel study from our laboratories[16]. Finally, an impressive indication is provided by **1h**, a quinolyl-penem wherein most of the activity of the quinolone

Table 2. Therapeutic efficacy of selected quinolyl-penems and reference compounds in mouse septicemias[a].

Microorganism	Therapy after infections (hours)	ED_{50} (mg/kg, cumulative dose)			
		1d	**1i**	FCE 22101	**2i**
Staphylococcus aureus ATCC 13709	2	3.8	1.99	0.87	1.12
Escherichia coli 5709 (β-lactamase+)	0.5~1.5~6	1.27	0.66	29.4	0.046
Pseudomonas aeruginosa ATCC 2598	1~3	6.3	3.27	>100	0.5

[a] Groups of 8~10 CD1 mice were infected by intraperitoneal route and treated subcutaneously according to the reported schedule. The mortality was recorded daily and ED_{50} calculated 5 days after infection.

component (norfloxacin) was intentionally suppressed by derivatization of the terminal nitrogen; this compound, as compared to the analogue **1g** incorporating the fully active quinolone, remains exquisitely potent against Gram-positive organisms.

The picture is much less clear as far as the activity on Gram-negative bacteria, *Pseudomonas* included, is concerned. Penems incorporating a poorly active component (**1a, 1b, 1c, 1h**) are also poorly active on these organisms. This result may be ascribed either to a low intrinsic activity (as a penem) of the intact molecule or to a poor penetration through the outer membrane (OM). A simple way to estimate the ability of the molecules to cross the OM of *Escherichia coli* is comparing their activity against *E. coli* UB 1005 and its OM-defective mutant DC2[17], which are equally susceptible to FCE 22101 (MIC=0.78 μg/ml). In the selected group of bifunctional penems incorporating a second component of negligible activity (**1a~1c, 1h**), with the exception of the cycloserine derivative, the susceptibility of the two strains differed considerably. Thus, these compounds seem to diffuse poorly through the OM of *E. coli*, either because of lipophilicity (**1a, 1b**) or for the high molecular weight and low flexibility of the molecule (quinolyl-penem **1h**). Unfortunately, for the other compounds, including the most interesting ones **1d** and **1i**, a reliable estimate is not possible, since their true activity might have been leveled to the observed values by the release in the culture medium of minor amounts of the highly active ciprofloxacin component. By inference, one could expect for the carbamate-linked compound **1d** (a dianion) a slow rate of diffusion through the important Omp F of *E. coli*[18], even slower than that of **1h** (a monoanion), while for the ester-linked analogue **1i** (a zwitterion) a reasonably fast diffusion is still possible. In this context the affinity of **1d** and **1i** for the essential penicillin-binding proteins (PBPs) of *E. coli* measured on membrane preparation confirmed the penem-like activity of the compounds (affinity for PBPs: 1a≥2>1b>3). However when the assay was performed on whole cells, only compound **1i** demonstrated the capability to reach the same essential PBPs, while **1d**, tested at up to 10×MIC concentration, still did not bind to the targets.

The remaining question about the whole molecule of quinolyl-penems is whether it can act as a quinolone *per se*. The ester-linked compounds (**1e~1f**), wherein the essential[19] carboxyl group of the quinolone component is not free, are not expected to display quinolone-like activity unless hydrolyzed. Also the carbamate-linked compounds cannot benefit from this type of contribution, since the carbamate link, suppressing the basicity of the piperazine terminal nitrogen atom, at the same time suppresses most of the potential quinolone-like activity of the intact molecule. In fact, inspection of Table 1 reveals that derivatization of norfloxacin (**2g**) as the simple ethyl carbamate (**2h**) is accompanied by a dramatic loss of activity against Gram-negative organisms.

Preliminary mouse protection tests (Table 2) seem to confirm for selected quinolyl-penems **1d** and **1i** the contribution of the dual mode of action observed *in vitro*. In particular **1i** (FCE 26600), even if almost insoluble in water, proved potent and virtually equi-effective in the treatment of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* systemic infections when given subcutaneously as a suspension. Its degradation pattern in human serum *ex vivo* (extrapolated half-life > 8 hours, with partial release of ciprofloxacin but not of the hydroxymethylpenem FCE 22056) confirms the unexpected stability of the ester link of quinolyl-penems, probably extending to *in vivo* conditions, with obvious implications for the potential interest of this class of compounds.

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ETTORE PERRONE
DANIELA JABÉS
MARCO ALPEGIANI
BIANCA PATRIZIA ANDREINI
COSTANTINO DELLA BRUNA
STEFANO DEL NERO
ROSARIA ROSSI
GIUSEPPINA VISENTIN
FRANCO ZARINI
GIOVANNI FRANCESCHI*

Farmitalia Carlo Erba, R. & D.,
Via dei Gracchi 35, Milano, Italy

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