SYNTHESIS AND BIOLOGICAL ACTIVITY OF C-3′ *ORTHO* DIHYDROXYPHTHALIMIDO CEPHALOSPORINS

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A series of C-3′ *ortho* dihydroxyphthalimido cephalosporins **3**~**7** has been prepared by reaction of C-3′ aminomethyl cephalosporin **41**[1] with the corresponding *N*-carboethoxyphthalimides **23**~**25**, **37**, **38**. These new caphalosporins exhibit excellent *in vitro* Gram-negative activities, including *Pseudomonas aeruginosa*, excellent β-lactamases stability and pharmacokinetics equivalent or better than ceftriaxone.

A number of new cephalosporins possess potent and extended antibacterial spectra and have made an important contribution to the treatment of infection[2]. However there is still a need for new semisynthetic cephalosporins with improved spectra, including *Pseudomonas aeruginosa*, increased β-lactamase stability (type I and plasmid mediated) and long half-lives.

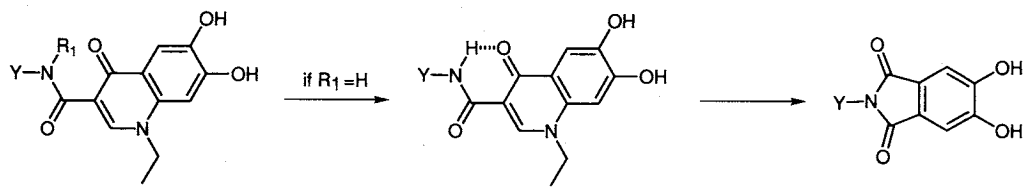
Since the discovery by Eisai of a C-7 ureido cephalosporin bearing a catechol moiety, E-0702[3], a new class of compounds, catechol containing cephalosporins, has emerged[4~6]. The catechol moiety predisposes the cephalosporins to pass into the bacterial cells not only passively *via* porins, the standard route of entry for cephalosporins, but also *via* an iron-uptake pathway[7,8].

During a programme to design modified cephalosporins, our laboratories have focused their interest on attaching a catechol moiety at the C-3′ position of the cephalosporin nucleus *via* an amidic linkage[1,9] such as in compound **1** (scheme 1).

We have found that such a combination leads to compounds having very good *in vitro* antibacterial activities, including *P. aeruginosa*, good type I β-lactamases stability and long half-lives[1,9].

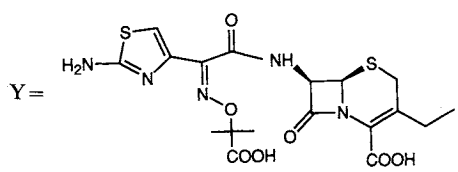
Particularly outstanding β-lactamase stability has been observed in compounds such as **1** and might

Scheme 1.



1 R_1 = H
2 R_1 = Et

3



be explained partly by the hydrogen bond (Scheme 1) observed between the amidic NH and the carbonyl of the quinolone ring (**1**: NMR δ 10.4 ppm in DMSO-d_6 compared to 8.3 ppm for normal amidic compounds) which result in a planar C-3′ substituent[1].

In order to understand the role played by the planar conformation of the C-3′ substituent and to further improve the type I β-lactamase stability, we decided to replace this labile hydrogen bond by a covalent one as in **3** (Scheme 1). The synthesis and biological properties of these new compounds are described in this paper.

Chemistry

The strategy used to prepare C-3′ *ortho* dihydroxyphthalimido cephalosporins[10] **3**~**7** involves *N*-carboethoxyphthalimides **23**~**25**, **37**, **38** as intermediates. Such reagents are known to react smoothly with amines to give the corresponding *N*-phthalimido protected compound in good yield[11]. Thus we synthesized the functionalised catechol phthalimides **23**~**25**, **37**, **38** and reacted them with the C-3′ aminomethyl cephalosporin **41**[1].

3,4-Dimethoxytoluene was formylated using dichloromethylmethyl ether in the presence of tin chloride to give the aldehyde **10** (Scheme 2). The brominated isomer **11** was obtained from vanillin after monobromination and similar formylation. **10**, **11** were then oxidized with $KMnO_4$ to give the diacids **12**, **13** which were transformed into the corresponding phthalimides **14**, **15** *via* the intermediate anhydrides. After deprotection of the catechol moiety using BBr_3, **17** was chlorinated using *N*-chlorosuccinimide and paratoluenesulfonic acid. In order to avoid side reactions during the *N*-carboethoxylation step, the catechol moiety was reprotected with an acid labile group; the most convenient proved to be the diphenylmethyl moiety. Protected phthalimides **20**~**22** were then reacted with NaH followed by ethyl chloroformate to give reagents **23**~**25**.

Condensation of **23**~**25** with **41** in DMSO occurs smoothly at room temperature and the protected cephalosporins **26**~**28** were isolated in very good yield. Final deprotection of the catechol moiety was achieved using trifluoroacetic acid in the presence of traces of water to give the free *ortho* dihydroxyphthalimido cephalosporins **3**~**5** (Scheme 2).

A similar strategy was used to generate the isomeric series, cephalosporins **6** and **7** (Scheme 3).

In addition, compound **8** (Table 1) used here as a comparator was obtained following a similar sequence starting from commercially available *N*-carboethoxyphthalimide.

Biological Results and Discussion

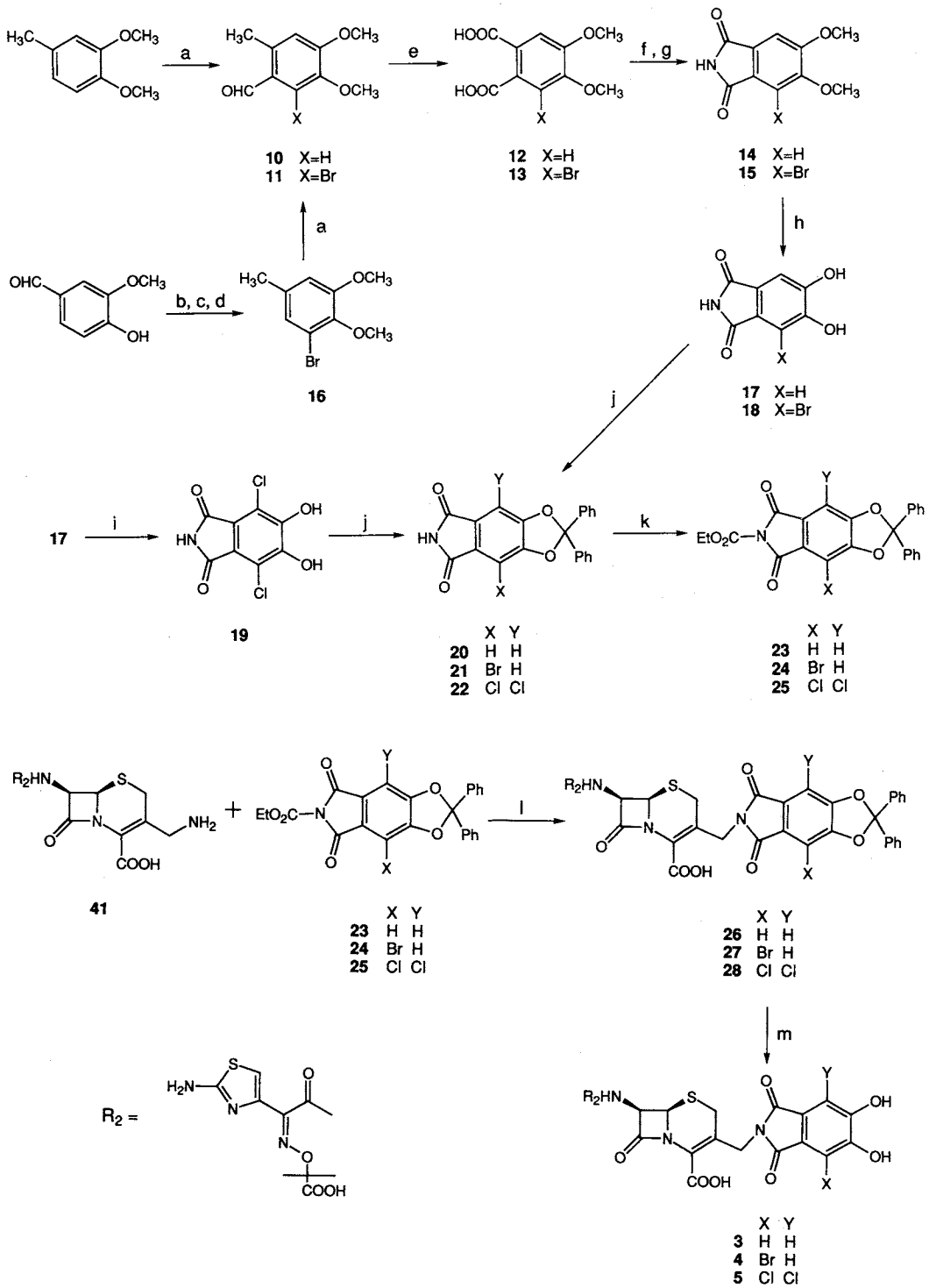
MIC values (μg/ml) of the C-3′ *ortho* dihydroxyphthalimido cephalosporins **3**~**7** against a selection of Gram-positive and Gram-negative bacteria are shown in Table 1. Compounds **3**~**7** have been compared with cephalosporin **1** and **2** (Scheme 1) and with classical standards.

A first look at Table 1 shows that activities of phthalimides **3**~**7** are superior to simple monocyclic catechols such as **9** (Table 1) having a phenolic p*Ka*$\approx$9, against *E. cloacae*, *P. stuartii*, *C. freudii*, *S. marcescens* and *P. morganii*. Against *P. aeruginosa* **3**~**7** are equivalent to **9**.

The level of activity against Gram-negative organisms obtained with the parent deshydroxyphthalimido cephalosporin **8**, compared with phthalimides **3**~**5** underlines clearly the importance of the *ortho*-dihydroxy function.

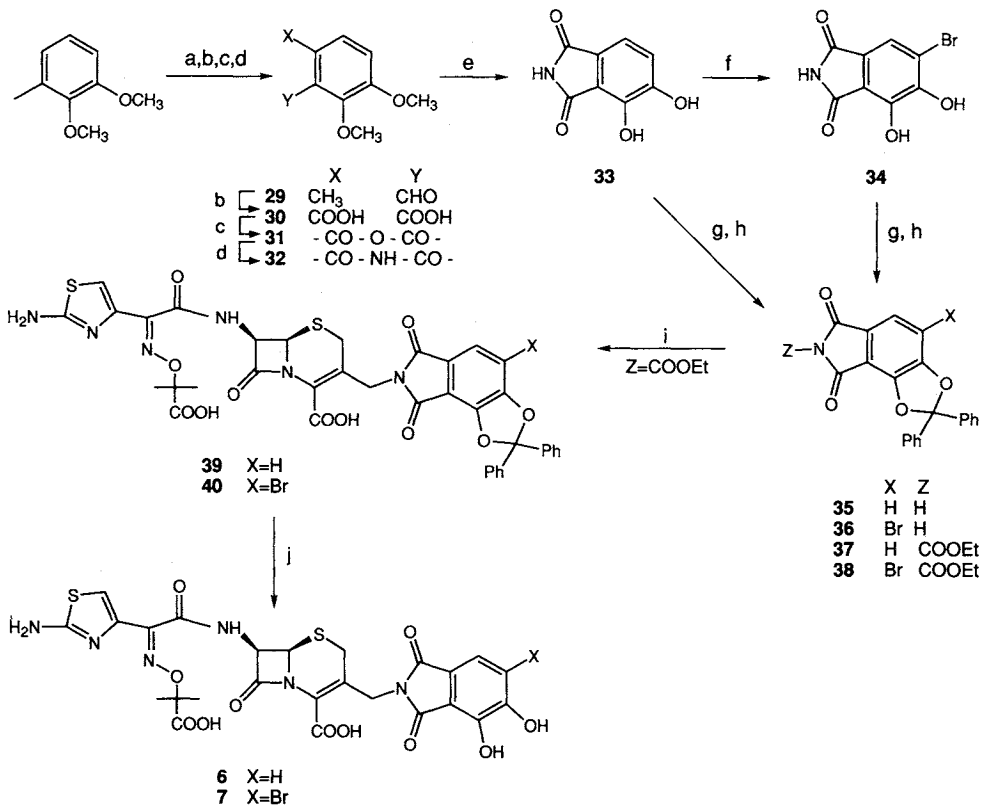
Comparison of biological activities against *P. aeruginosa* 799/61 and *Escherichia coli* DC2, the permeability mutants lacking the outer membrane of 799WT and DC0, respectively, shows that **3**~**5**

Scheme 2. Synthesis of cephalosporines **3**, **4**, and **5**.



a) Cl_2CHOCH_3, $SnCl_4$, b) Br_2, CH_3COOH, c) $(CH_3)_2SO_4$, K_2CO_3, d) 1) NH_2NH_2, 2) KOH, e) $KMnO_4$, K_2CO_3, f) AC_2O, g) NH_4OH, h) BBr_3, i) *N*-chlorosuccinimide, paratoluenesulfonic acid, j) Ph_2CCl_2 160°C, k) 1) NaH, DMF, 2) $ClCO_2Et$, l) Et_3N, DMSO, m) TFA, H_2O.

Scheme 3. Synthesis of cephalosporines **6** and **7**.



a) Cl_2CHOCH_3, $SnCl_4$, b) $KMnO_4$, K_2CO_3, c) Ac_2O, d) NH_4OH, e) BBr_3, CH_2Cl_2, f) Br_2, CH_3COOH, g) Ph_2CCl_2, h) 1) NaH, THF, 2) $ClCO_2Et$, i) **41**, Et_3N, DMSO, j) TFA, H_2O.

(MIC's: 0.001 ~ 0.008 μg/ml) and the parent **8** (MIC: 0.008 μg/ml) have similar intrinsic activity. However comparison of activities against *P. aeruginosa* 799WT and *E. coli* DC0 and their permeability mutants 799/61 and DC2, respectively shows that molecules **3**~**5** bearing a catechol residue at C-3′ penetrate well into bacteria (ratio 799WT/799/61: **3**~**5**=1 to 2; Ceftazidime =30) while **8** is much less efficient (ratio 799WT/799/61=1,000). The result of passive diffusion is well represented by the activity of **8** against *P. aeruginosa* 799WT (MIC=8 μg/ml).

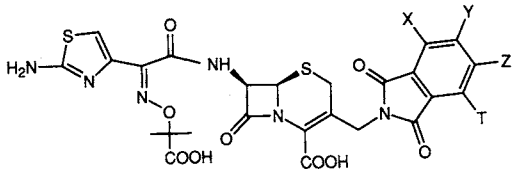
This clearly indicates that the catechol moiety facilitates the penetration through the outer membrane of bacteria.

The mechanism of penetration has been shown to be *ton* B dependent[8], the known functionality of the *ton* B gene product concerning the transport of iron-chelated siderophores in *E. coli*.

Against Gram-positive organisms **3**~**5** are less active than cefpirome and cefepime. Against *Streptococcus pneumoniae* and *Streptococcus pyogenes* **4** and **5** are just equivalent to ceftazidime, **4** being the best compound.

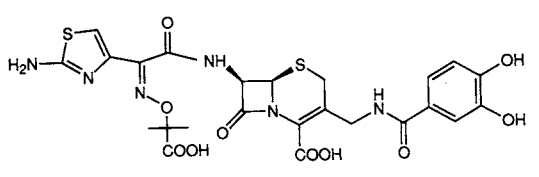
Against inducible and derepressed type I β-lactamase-producers (see Table 1, footnotes b, e) *P. aeruginosa*, *Enterobacter cloacae*, *Providencia stuartii*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus morganii* and *Klebsiella oxytoca*, C-3′ *ortho* dihydroxyphthalimido cephalosporins **3**~**7** are superior to

Table 1. Antibacterial properties of C-3′ *ortho* dihydroxyphthalimido cephalosporins comparison with standards (MIC's μg/ml)[a].



	3	4	5	6	7	8
X	H	Br	Cl	H	H	H
Y	OH	OH	OH	H	Br	H
Z	OH	OH	OH	OH	OH	H
T	H	H	Cl	OH	OH	H

9



Organisms	Compounds											
	3	4	5	6	7	8	1	2	Ceftazidime	Cefpirome	Cefepime	9
Pseudomonas aeruginosa												
18S[e]	≤0.008	0.004	0.004	≤0.008	≤0.008	16	≤0.008	0.015	2	8	4	0.008
18SSAI[f]	≤0.008	0.004	0.008	≤0.008	≤0.008	16	≤0.008	0.015	1	2	1	0.008
18SSH[b]	≤0.008	0.004	0.004	≤0.008	≤0.008	8	≤0.008	0.125	32	32	16	0.06
50	≤0.008	0.008	0.008	0.008	≤0.008	2	0.015	0.015	2	1	0.5	0.008
50DR	≤0.008	0.015	0.015	0.008	≤0.008	8	0.03	0.125	32	16	8	0.125
799WT[c]	≤0.008	0.001	0.002	≤0.008	≤0.008	8	≤0.008	0.015	0.25	0.125	0.06	0.008
799/61[d]	≤0.008	0.002	0.004	≤0.008	≤0.008	≤0.008	≤0.008	0.015	≤0.008	≤0.008	≤0.008	0.008
PU21	≤0.008	0.008	0.015	≤0.008	≤0.008	16	0.03	0.03	1	1	1	—
Enterobacter cloacae												
P99^{-}[e]	≤0.008	0.06	0.03	0.03	0.015	2	0.06	0.125	0.125	0.06	0.03	0.5
P99^{+}[b]	0.03	0.125	0.06	0.06	0.06	16	0.06	1	32	0.5	0.25	16
I^{+} 401029[e]	0.015	0.015	0.015	≤0.008	0.015	1	0.06	0.06	0.125	0.03	0.03	0.25
DR 401108[b]	0.5	0.5	0.25	0.25	1	8	1	2	32	0.25	0.125	>128

Providencia stuartii												
I^+ 442019[e]	≤0.008	0.004	0.004	0.015	0.06	2	0.25	1	0.25	0.125	0.06	0.125
DR 442049[b]	0.125	0.015	0.015	0.25	0.5	4	8	16	4	0.5	0.125	1
Citrobacter freundii												
I^+ 382010[e]	≤0.008	0.001	0.001	≤0.008	≤0.008	1	≤0.008	≤0.008	0.125	0.015	0.015	0.03
DR 382031[b]	0.06	0.125	0.06	0.125	0.125	32	0.125	1	128	1	0.5	32
Serratia mercescens												
I^+ 421003[e]	≤0.008	0.004	0.004	≤0.008	≤0.008	4	0.015	0.03	0.25	0.06	0.06	0.06
DR 421078[b]	≤0.008	0.004	0.004	≤0.008	≤0.008	4	0.015	0.125	0.5	0.06	0.125	0.06
Proteus morganii												
I^+ 433001[e]	≤0.008	0.004	0.004	≤0.008	≤0.008	1	0.125	0.5	0.125	0.03	0.03	0.125
DR 433062[b]	0.03	0.03	0.015	0.06	0.03	4	1	8	8	0.125	0.03	8
Klebsiella oxytoca												
$K1^+$ 395056[e]	≤0.008	0.002	0.004	≤0.008	≤0.008	—	≤0.008	—	0.5	1	1	—
$K1^-$ 395055	≤0.008	0.001	0.004	≤0.008	≤0.008	—	≤0.008	—	0.06	—	0.008	0.015
Escherichia coli												
DC0[c]	≤0.008	0.001	0.002	≤0.008	≤0.008	1	≤0.008	≤0.008	0.125	0.015	0.015	0.008
DC2[d]	≤0.008	0.001	0.001	≤0.008	≤0.008	0.06	≤0.008	≤0.008	0.06	0.015	0.015	0.008
Staphylococcus aureus												
Oxford[f]	16	16	8	16	8	8	16	32	4	0.125	0.25	8
147N[f]	16	16	16	16	16	8	16	64	4	0.25	0.5	16
Streptococcus pneumoniae												
671001	—	0.125	0.25	—	0.5	0.25	0.25	0.25	0.125	0.015	0.015	0.125
Streptococcus pyogenes												
681018	—	0.25	0.5	0.5	1	0.06	0.25	1	0.125	≤0.008	≤0.008	—

[a] IST growth medium, inoculum 10^4 cfu per spot, 37°C; [b] constitutive derepressed type I β-lactamase-producer; [c] parent organism; [d] permeability mutant; [e] inducible type I β-lactamase producer; [f] non inducible mutant; [g] penicillin-sensitive *S. aureus*.

Table 2. Pharmacokinetics measured in marmosets following intravenous administration of a 3 mg/kg dose.

Compound	AUC* (mg/hour per liter)	Half-life* (hours)	Conc.* (at 3 hours) (mg/liter)	Measured p*Ka*
3	18.2	0.66	0.9	7.1
4	113～152	1.8～3.3	14.2～18.0	5.9
5	62.6	1.5	8.1	5.3
6	36.4	1.3	4.4	6.3
7	80.9	2.0	8.0	5.3
Ceftriaxone	75.7	1.5	8.8	—

* Measured by bioassay.

ceftazidime. **3**～**7** also proved to be 2 to 100 times more active against constitutive derepressed and inducible type I β-lactamases-producing strains than cefepime and cefpirome (Table 1).

Comparison of mic's against β-lactamase-derepressed producers (Table 1 footnote b) and the corresponding β-lactamase-inducible producers (Table 1 footnote e) indicates that **3**～**7** are more stable to β-lactamases than the monocyclic catechol and than ceftazidime (*E. cloacae* $P99^+/P99^-$: **3**～**7**: ratio = 2～4, **9**: ratio = 32, ceftazidime: ratio = 256; *C. freundii* DR382031/I^+382010: **3**～**7**: ratio = 7.5～125, **9**: ratio = 1,067, ceftazidime: ratio = 1,024). Similar comparisons indicates (Table 1) that C-3′ *ortho* dihydroxyphthalimido cephalosporins **3**～**7** are marginally more stable to β-lactamases than cefepime and cefpirome, the accepted type I "stable cephalosporins", against *E. cloacae* (comparison $P99^+/P99^-$) and *C. freundii* (I^+382010/DR382031) but are very similar against *E. cloacae* (comparison I^+401029/DR401108), *P. stuartii*, *S. marcescens* and *P. morganii* (see Table 1).

Overall the activity and stability against constitutive and inducible derepressed type I β-lactamase-producing strains has been improved. These results showed clearly that the stability observed in compounds possessing an H-bonding between the NH of the C-3′ amidic linkage and the carbonyl group of the quinolone moiety (Scheme 1) (comparison of **1** and **2**) has been reinforced in phthalimido catechol cephalosporin such as **3**. Furthermore the bulk of C-3′ phthalimido substituents as well as the acidity of the OH of the *ortho*-dihydroxy moiety reinforce the observation that hindrance at C-3′ and acidity of the catechol[1,9] improves the β-lactamase stability.

The pharmacokinetics of **5** and **7** measured in the marmoset (Table 2) were equivalent to those of ceftriaxone, an injectable cephalosporin suitable for once-daily dosing. **4** is clearly superior as measured by either half-life or the elevation of serum level which results in higher AUC's. As previously observed[9] these pharmacokinetic parameters seem related to the p*Ka* of the catechol moiety: molecules highly ionised at physiological pH have the longest pharmacokinetics (Table 2 comparison of **3** with **4** and **5** and comparison of **6** with **7**). Nevertheless comparison of **4** and **5** (Table 2) indicates that additionnal factors can modulate the pharmacokinetics of such compounds. Amongst them we could probably suspect the serum protein binding and the ability of catechol-cephalosporins to be metabolized by the enzyme catechol-*O*-methyltransferase (COMT)[12].

Urinary recoveries (measured in marmosets, single iv dose of 3 mg/kg) of these compounds are equivalent to ceftriaxone; values of ≈25% suggest a significant degree of biliary excretion. All three compounds are highly found to Human serum protein (measured at 50 mg/liter), ranking from 94% for **3** (equivalent to ceftriaxone) to 99.6% for **4** and 98% for **5**.

Experimental

IR spectra were recorded as KBr pellets on a Perkin-Elmer 781 spectrophotometer. ^{1}H NMR were recorded on a JEOL FX90Q or a Brucker 300AC spectrometer. FAB mass spectra were obtained on a VG 7250 SA mass spectrometer. Analytical HPLC chromatography was carried out on Shimadzu LC6A apparatus using nucleosil C18 5 μm columns, eluent MeOH - H_2O with 1 : 100 AcOH. Preparative medium pressure chromatography was carried out using Mitsubishi HP20SS resin, eluent MeOH - H_2O with 1 : 100 AcOH. C-3'-Aminomethyl cephalosporin **41** was described in the literature[1,13].

4,5-Dimethoxy-2-methylbenzaldehyde (**10**)

To a solution of 3,4-dimethoxytoluene (15 g, 98.6 mmol) and dichloromethylmethyl ether (34.5 g) in dichloroethane (200 ml), at 0°C, was added $SnCl_4$ (100 ml) and, subsequently, further dichloroethane (200 ml). The resultant suspension was stirred at 0°C for 20 minutes and then stirred overnight at ambient temperature before being poured into 3 N HCl (500 ml) at 0°C. Extraction into dichloromethane, drying of the organic phase and evaporation gave 4,5-dimethoxy-2-methylbenzaldehyde[14] (15.2 g); ^{1}H NMR ($CDCl_3$) δ 2.6 (s, 3H), 3.90 (s, 3H), 3.94 (s, 3H), 6.68 (s, 1H), 7.3 (s, 1H), 10.2 (s, 1H); CI-MS *m/z* 181 ($M+H^+$, $C_{10}H_{13}O_3$); mp 76~78°C.

Anal Calcd for $C_{10}H_{12}O_3$: C 66.65, H 6.71.
Found C 66.62, H 6.98.

11, **29**[15] were prepared in a similar way:

11: yield 98%; ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H), 3.77 (s, 3H), 3.9 (s, 3H), 7.0 (s, 1H), 10.3 (s, 1H); IR ν_{max} cm^{-1} 1680, 1595; CI-MS *m/z* 259, 261 ($M+H^+$, $C_{10}H_{12}O_3Br$); mp 114~115°C.

Anal Calcd for $C_{10}H_{11}O_3Br$: C 46.33, H 4.25.
Found C 46.57, H 4.46.

29: yield 70%; ^{1}H NMR (DMSO-d_6, CF_3COOD) δ 2.45 (s, 3H), 3.7 (s, 3H), 3.85 (s, 3H), 7.0 (d, 1H), 7.55 (d, 1H), 9.99 (s, 1H); IR ν_{max} cm^{-1} 1690, 1600. A sample recrystallized twice from petroleum ether gave a melting point of 52~54°C (lit.[15] mp 53.5~54.5°C).

2-Carboxy-4,5-dimethoxybenzoic Acid (**12**)

To aldehyde **10** (5 g, 44.4 mmol) and potassium carbonate (5 g) in water (21 ml) at 80°C was added, in one portion, a solution of potassium permanganate (19 g) in water (170 ml). The solution was heated at 90~95°C for 1 hour, cooled, neutralized with 5 N HCl (50 ml) and filtered through diatomaceous earth. The aqueous phase was concentrated and extracted into ethyl acetate; the organic phase was dried and evaporated to give 2-carboxy-4,5-dimethoxybenzoic acid (2.85 g); ^{1}H NMR ($CDCl_3$ - DMSO-d_6) δ 3.95 (s, 6H), 7.5 (s, 2H), 11.2 (broad s, 2H), mp 196~198°C (lit.[16] mp 198~199.5°C).

Diacids **13**, **30** were prepared in a similar way:

13: yield 25.6%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 3.85 (s, 3H), 3.95 (s, 3H), 7.55 (s, 1H); IR ν_{max} cm^{-1} 3400~2400, 1720~1700, 1580; mp 194~196°C.

Anal Calcd for $C_{10}H_9O_6Br$: C 39.36, H 2.95.
Found C 39.74, H 3.14.

30: yield 27%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 3.74 (s, 3H), 3.9 (s, 3H), 7.1 (d, 1H), 7.7 (d, 1H); IR ν_{max} cm^{-1} 3750~3200, 1700.

3,4-Dimethoxyphthalimide (**14**)

The di-acid **12** (1.5 g, 6.6 mmol) and acetic anhydride (6 g) were heated at reflux for 1 hour. The mixture was cooled and evaporated to provide dimethoxyphthalic anhydride (1.28 g); ^{1}H NMR (DMSO-d_6) δ 3.99 (s, 6H), 7.6 (s, 2H).

To the crude anhydride (2.0 g, 9.6 mmol) was added 28% ammonia solution (2.3 g) and the solution was taken to boiling-point, eliminating water in order to obtain a thick paste.

This was cooled to give a brown solid which was finely ground. This solid was heated by a flame

(without fusion) to give dimethoxyphthalimide[16] (1.7 g). The reaction was monitored by HPLC. **14** was recristallized using EtOH - petroleum ether. ^{1}H NMR (DMSO-d_6) δ 3.9 (s, 6H), 7.3 (s, 2H), 10.9 (s, 1H); CI-MS m/z 225 ($M+NH_4^+$); mp > 320°C (lit.[16] mp > 300°C).

Anal Calcd for $C_{10}H_9NO_4$: C 57.97, H 4.34, N 6.76.
Found C 57.73, H 4.55, N 6.78.

Phthalimides **15**, **32** were prepared in a similar way. Intermediate anhydrides were used crude without purification.

15: 1) anhydride: yield 100%; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 4.1 (s, 3H), 7.75 (s, 1H); 2) **15**: yield 100%; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 4.0 (s, 3H), 7.47 (s, 1H), 11.25 (m, 1H); IR ν_{max} cm^{-1} 3400~3100, 1770, 1740~1710, 1595; CI-MS m/z 285, 287 (M, $C_{10}H_8NO_4Br$).

32: 1) anhydride **31**: yield 100%; ^{1}H NMR (DMSO-d_6) δ 3.98 (s, 3H), 4.05 (s, 3H), 7.6 (d, 1H), 7.8 (d, 1H); IR ν_{max} cm^{-1} 1780, 1850; 2) **32**: yield 50%; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 3.97 (s, 3H), 7.35 (d, 1H), 7.52 (d, 1H), 11.0 (s, 1H); IR ν_{max} cm^{-1} 3200, 1765, 1740~1710, 1600; mp (EtOH) 225~228°C[17]; CI-MS m/z 207 (M, $C_{10}H_9NO_4$).

3,4-Dihydroxyphthalimide (**17**)

To the dimethoxyphthalimide **14** (3.1 g, 14.9 mmol) were added BBr_3 (12 ml) and dichloromethane (30 ml). The suspension was stirred at ambient temperature until HPLC showed that starting material had disappeared. Excess solvent was evaporated and the resultant solid cooled to 0°C and treated with ice and then with water (80 ml). The mixture was stirred for 45 minutes at ambient temperature, water (40 ml) removed by evaporation and the residue purified by column chromatography to provide dihydroxyphthalimide **17** (2.13 g); ^{1}H NMR (DMSO-d_6) δ 7.1 (s, 2H), 10.25 (br s, 2H), 11.25 (br s, 1H).

Dihydroxyphthalimides **18**, **33** were prepared in a similar way: **18**: yield 95%; ^{1}H NMR (DMSO-d_6) δ 7.1 (s, 1H); IR ν_{max} cm^{-1} 3600~2900, 1760, 1720~1700.

Anal Calcd for $C_8H_4NO_4Br$: C 37.23, H 1.56, N 5.43.
Found C 37.59, H 1.68, N 5.20.

33: yield 68%; ^{1}H NMR (DMSO-d_6) δ 7.1 (m, 2H), 10.75 (m, 1H); IR ν_{max} cm^{-1} 3500~2900, 1760, 1720; EI-MS m/z 179 (M, $C_8H_5NO_4$).

3,4-Diphenylmethylenedioxyphthalimide (**20**)

A suspension of the dihydroxyphthalimide **17** (350 mg, 19 mmol) in diphenyldichloromethane (4 g) was heated at 160°C for 3 hours. The resultant solution was cooled and washed with petroleum ether (3 × 10 ml). The petrol phase was separated to give a brown oil which was collected and triturated under diethyl ether (5 ml) to give as a chestnet brown solid, diphenylmethylenedioxyphthalimide **20** (300 mg); ^{1}H NMR (DMSO-d_6) 7.5 (m, 12H), 11.05 (s, 1H); CI-MS m/z 344 ($M+H^+$, $C_{21}H_{14}NO_4$); mp 170~173°C.

Anal Calcd for $C_{21}H_{13}NO_4$: C 73.47, H 3.79, N 4.08.
Found C 73.03, H 4.05, N 3.96.

Cooling of the petrol phase afforded a second fraction of the phthalimide (250 mg).

21 and **22** were recristallized from EtOH - petroleum ether.

Phthalimides **35**, **36**, **21** and **22** were prepared in a similar way:

21: yield 62.5%; ^{1}H NMR (DMSO-d_6) δ 7.5 (s, 1H), 7.55 (s, 10H); IR ν_{max} cm^{-1} 3400~3100, 1780, 1715; mp 194~196°C; CI-MS m/z 422, 424 ($M+H^+$, $C_{21}H_{13}NO_4Br$).

Anal Calcd for $C_{21}H_{12}NO_4Br$: C 59.71, H 2.84, N 3.31.
Found C 59.55, H 2.96, N 3.35.

22: yield 58%; ^{1}H NMR (DMSO-d_6) δ 7.5 (s, 10H); IR ν_{max} cm^{-1} 3200, 1770, 1720; white plates mp 230~232°C; CI-MS m/z 412, 414 ($M+H^+$, $C_{21}H_{12}NO_4Cl_2$).

Anal Calcd for $C_{21}H_{11}NO_4Cl_2$: C 61.16, H 2.70, N 3.42.
Found C 61.23, H 2.89, N 3.39.

35: yield 49%; ^{1}H NMR (DMSO-d_6) δ 7.4 (s, 2H), 7.55 (s, 10H), 11.2 (s, 1H); IR ν_{max} cm^{-1} 1770,

1720, 1700; CI-MS *m/z* 343 (M, $C_{21}H_{13}NO_4$).

36: yield 80%; ^{1}H NMR (DMSO-d_6) δ 7.55 (s, 11H), IR ν_{max} cm^{-1} 1770, 1720.

Anal Calcd for $C_{21}H_{12}NO_4Br$: C 59.71, H 2.84, N 3.31.
Found C 59.84, H 3.04, N 3.30.

N-Carboethoxy-3,4-diphenylmethylenedioxyphthalimide (**23**)

To a suspension of sodium hydride (4 mg, 50%) (washed with tetrahydrofuran) was added, dropwise, a solution of phthalimide **20** (500 mg, 1.45 mmol), in DMF (1.5 ml). The mixture was stirred for 1 hour at ambient temperature, cooled to 0°C and ethyl chloroformate (180 μl) was added dropwise. The resultant solution was stirred at 0°C for 5 minutes, stirred at ambient temperature for 3 hours, cooled to 0°C and water (5 ml) added with stirring. The solution was extracted with diethyl ether (150 ml). The ether phase was washed with water (3×20 ml), saturated NaCl (20 ml), dried, filtered and evaporated to give *N*-carboethoxy diphenylmethylenedioxyphthalimide (600 mg); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H), 4.45 (q, 2H), 7.25~7.75 (m, 12H).

N-Carboethoxyphthalimides **24**, **25**, **37** and **38** were prepared in a similar way. Compounds **23**, **24**, **38** decomposes partially when purified over silica or HP20SS resines and have thus been used crude in the next step.

24: yield 95%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 1.3 (t, 3H), 4.3 (q, 2H), 7.25~7.75 (m, 11H).

25: yield 100%; ^{1}H NMR (DMSO-d_6) δ 1.3 (t, 3H), 4.35 (q, 2H), 7.55 (s, 10H); IR ν_{max} cm^{-1} 1680. Recrystallized 3 times from EtOH. mp 158~160°C; CI-MS *m/z* 484, 486 ($M+H^+$, $C_{24}H_{16}NO_6Cl_2$).

Anal Calcd for $C_{24}H_{15}NO_6Cl_2$: C 59.50, H 3.10, N 2.89.
Found C 59.48, H 3.30, N 2.94.

37: yield 74.2%; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 4.45 (q, 2H), 7.2~7.7 (m, 12H); IR ν_{max} cm^{-1} 1800, 1780, 1720; CI-MS *m/z* 415 (M, $C_{24}H_{17}NO_6$).

38: yield 81.5%; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3H), 4.4 (q, 2H), 7.55 (s, 11H); IR ν_{max} cm^{-1} 1800, 1760, 1720.

7-[2-(2-Aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-(5,6-diphenylmethylenedioxy-1,3-dioxo-isoindol-2-ylmethyl)ceph-3-em-4-carboxylic Acid (**26**)

To a solution of 7-[2-(2-aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-aminomethylceph-3-em-4-carboxylic acid **41** (143 mg) in DMSO (2 ml) was added triethylamine (62.4 mg) followed by the phthalimide **23** (85.6 mg, 0.21 mmol) in DMSO (1 ml).

The solution was stirred at ambient temperature for 90 minutes, concentrated HCl (10 drops) added and after storage at 3°C for 12 hours the solution was evaporated and purified by column chromatography using HP20SS resin to give **26** (70 mg); ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 1.5 (2s, 6H), 3.3 (d, 1H), 3.6 (d, 1H), 4.4 (d, 1H), 4.90 (d, 1H), 5.1 (d, 1H), 5.8 (d, 1H), 7.05 (s, 1H), 7.25~7.75 (m, 12H).

27, **28**, **39** and **40** were prepared in a similar way:

27: yield 61%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 1.5 (2s, 6H), 3.25 (dAB, 1H), 3.7 (dAB, 1H), 4.35 (dAB, 1H), 4.9 (dAB, 1H), 5.1 (d, 1H), 5.85 (d, 1H), 7.05 (s, 1H), 7.25~7.75 (m, 1H); IR ν_{max} cm^{-1} 3700~2700, 1770, 1710; FAB-MS *m/z* 889, 891 ($M+H^+$, $C_{38}H_{30}N_6O_{11}S_2Br$).

28: yield 63.1%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 1.5 (2s, 6H), 3.3 (dAB, 1H), 3.65 (dAB, 1H), 4.4 (dAB, 1H), 4.9 (dAB, 1H), 5.1 (d, 1H), 5.85 (d, 1H), 7.05 (s, 1H), 7.55 (s, 10H); FAB-MS *m/z* 879, 881 ($M+H^+$, $C_{38}H_{29}N_6O_{11}S_2Cl_2$).

39: yield 59.5% (not purified; used crude in the next step).

40: yield 69%; ^{1}H NMR (DMSO-d_6) δ 1.54 (s, 3H), 1.56 (s, 3H), 3.3 (dAB, 1H), 3.65 (dAB, 1H), 4.4 (dAB, 1H), 4.9 (dAB, 1H), 5.1 (d, 1H), 5.85 (d, 1H), 7.05 (s, 1H), 7.5 (s, 10H), 7.65 (s, 1H); IR ν_{max} cm^{-1} 1770, 1710; FAB-MS *m/z* 889, 891 ($M+H^+$, $C_{38}H_{30}N_6O_{11}S_2Br$).

7-[2-(2-Aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-(5,6-dihydroxy-1,3-dioxo-isoindol-2-ylmethyl)ceph-3-em-4-carboxylic Acid (**3**)

To 7-[2-(2-aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-(5,6-diphenylmethylenedioxy-1,3-dioxo-isoindol-2-ylmethyl)ceph-3-em-4-carboxylic acid (**26**) (70 mg) was added tri-

fluoroacetic acid (4 ml) followed by water (2 drops).

The solution was stirred for 4 hours at ambient temperature. The solvent was removed by evaporation and the solid residue was diluted with dimethylformamide (3 ml) and subjected to column chromatography using HP20SS resin to afford the title compound (30 mg); ^{1}H NMR (DMSO-d_6 - CD_3COOD - CF_3COOD) δ 1.5 (2s, 6H), 3.25 (d, 1H), 3.55 (d, 1H), 4.35 (d, 1H), 4.8 (d, 1H), 5.1 (d, 1H), 5.8 (d, 1H), 7.05 (s, 1H), 7.1 (s, 2H); FAB-MS m/z 647 (M+H); IR ν_{max} cm^{-1} 1755, 1700.

4, **5**, **6** and **7** were prepared in a similar way:

4: yield 66%; ^{1}H NMR (DMSO-d_6 - CF_3COOD - CD_3COOD) δ 1.5 (2s, 6H), 3.25 (dAB, 1H), 3.55 (dAB, 1H), 4.35 (dAB, 1H), 4.9 (dAB, 1H), 5.1 (dAB, 1H), 5.8 (dAB, 1H), 7.05 (s, 1H), 7.15 (s, 1H); IR ν_{max} cm^{-1} 3700~3400, 1770, 1700; FAB-MS m/z 723 (M−H).

5: yield 38%; ^{1}H NMR (DMSO-d_6 - CF_3COOD - CD_3COOD) δ 1.5 (2s, 6H), 3.3 (dAB, 1H), 3.6 (dAB, 1H), 4.35 (dAB, 1H), 4.9 (dAB, 1H), 5.1 (d, 1H), 5.8 (d, 1H), 7.05 (s, 1H); IR ν_{max} cm^{-1} 1760, 1700; FAB-MS m/z 715 (M+H).

6: yield 66%; ^{1}H NMR (DMSO-d_6 - CF_3COOD - CD_3COOD) δ 1.52 (s, 6H), 3.35 (d, 1H), 3.6 (d, 1H), 4.35 (dAB, 1H), 4.9 (dAB, 1H), 5.15 (d, 1H), 5.8 (d, 1H), 7~7.25 (m, 3H); IR ν_{max} cm^{-1} 1760, 1700; FAB-MS m/z 645 (M−H).

7: yield 61%; ^{1}H NMR (DMSO-d_6 - CF_3COOD - CD_3COOD) δ 1.53 (2s, 6H), 3.3 (dAB, 1H), 3.6 (dAB, 1H), 4.4 (dAB, 1H), 4.95 (dAB, 1H), 5.15 (d, 1H), 5.8 (d, 1H), 7.05 (s, 1H), 7.45 (s, 1H); IR ν_{max} cm^{-1} 1760, 1700, 1630; FAB-MS m/z 723 (M−H).

7-[2-(2-Aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-(1,3-dioxo-isoindol-2-ylmethyl)ceph-3-em-4-carboxylic Acid (**8**)

To 7-[2-(2-aminothiazol-4-yl)-2-((*Z*)-1-carboxy-1-methylethoxyimino)acetamido]-3-aminomethyl-ceph-3-em-4-carboxylic acid (200 mg) in DMSO (2 ml) was added triethylamine (55 mg, 0.56 mmol) followed by *N*-carboethoxyphthalimide (41 mg, 0.186 mmol). The solution was stirred at ambient temperature for 6 hours, concentrated HCl (6 drops) added and after storage at 3°C for 12 hours, the solution was evaporated and purified by column chromatography using HP20SS resin to afford the title compound (30 mg); ^{1}H NMR (DMSO-d_6 - CF_3COOD - CD_3COOD) δ 1.5 (s, 6H), 3.35 (d, 1H), 3.65 (d, 1H), 4.45 (d, 1H), 5.0 (d, 1H), 5.15 (d, 1H), 5.85 (d, 1H), 7.05 (s, 2H), 7.85 (s, 4H); FAB-MS m/z 613 (M−H).

3-Bromo-4,5-dimethoxytoluene (**16**)

1) Bromine (16 g, 0.1 mol) was added dropwise to a solution of vanillin (15.2 g, 0.1 mol) in acetic acid (66 ml) and the mixture stirred 12 hours at ambient temperature. The resulting solid was filtered and washed with ethanol to give bromovanillin (16.5 g); yield: 72%; ^{1}H NMR (DMSO-d_6) δ 3.9 (s, 3H), 7.3 (s, 1H), 7.6 (s, 1H), 9.7 (s, 1H); IR ν_{max} cm^{-1} 3500~3000, 1680.

2) To a solution of bromovanillin (8 g, 34.6 mmol) in DMF (65 ml) was added potassium carbonate (9.7 g, 69.2 mmol) followed by dimethylsulfate (5 ml, 51.9 mmol). Stirring was continued 3 days at ambient temperature after which water (400 ml) was added. The precipitate was filtered and recrystallized (petroleum ether) to afford 3-bromo-4,5-dimethoxybenzaldehyde (6 g); yield: 70%; ^{1}H NMR (DMSO-d_6 - CF_3COOD) δ 3.87 (s, 3H), 3.94 (s, 3H), 7.5 (s, 1H), 7.76 (s, 1H), 9.9 (s, 1H).

Anal Calcd for $C_9H_9O_3Br$: C 44.09, H 3.70.
Found C 44.36, H 3.81.

3) A solution of 3-bromo-4,5-dimethoxybenzaldehyde (3 g, 12.2 mmol) and hydrazine hydrate (15 g) in diethylene glycol (25 ml) was heated at 90~100 °C for 45 minutes. After cooling to ambient temperature KOH (1 g) was added and the solution was heated at 140~160 °C for 3 hours. After cooling the solution was poured into water (50 ml) and extracted with ether; the organic layer was washed with brine and dried ($MgSO_4$) to give the title compound **16**† as an oil (1.69 g); yield: 60%; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 3.7 (s, 3H), 3.8 (s, 3H), 6.9 (s, 1H), 6.97 (s, 1H); IR ν_{max} cm^{-1} 1600.

† NMR given in DMSO-d_6 for consistency—A sample ran in CCl_4 gave identical results to literature 18.

2,3-Dihydroxy-4-bromophthalimide (**34**)

A solution of phthalimide **33** (0.26 g, 1.4 mmol) and bromine (0.23 g, 1.4 mmol) in acetic acid (13 ml) was stirred 3 hours at 50°C. After 3 hours a further portion of bromine (0.23 g) in acetic acid (3 ml) was added and heating continued for 1 hour at 50°C and 3 hours at 80°C. After cooling and filtration the precipitate was washed with acetic acid and dried to give **34** (350 mg); yield: 100%; ^{1}H NMR (DMSO-d_6) δ 7.4 (s, 1H); IR ν_{max} cm^{-1} 1750, 1700.

Anal Calcd for $C_8H_4NO_4Br$: C 37.21, H 1.55, N 5.42.
Found C 37.51, H 1.70, N 5.24.

2,5-Dichloro-3,4-dihydroxyphthalimide (**19**)

To a suspension of **17** (0.4 g, 2.2 mmol) in acetic acid (12 ml) was added *N*-chlorosuccinimide (0.61 g, 4.6 mmol). After stirring 6 minutes at ambiant temperature paratoluenesulfonic acid (10 mg, 0.05 mmol) was added and the mixture heated at 60°C for 6 hours. After cooling, the precipitate was filtered off and dried overnight under vaccum (0.52 g); yield: 94%; ^{1}H NMR (DMSO-d_6, CF_3COOD) δ: No signals; CI-MS *m/z* 247, 249, 251 (M, $C_8H_3NO_4Cl_2$).

Anal Calcd for $C_8H_3NO_4Cl_2$: C 38.71, H 1.21, N 5.65.
Found C 38.95, H 1.37, N 5.50.

Conclusion

Introduction of an *ortho* dihydroxyphthalimido residue at C-3′ of the cephalosporin nucleus has led to compounds possessing exceptional *in vitro* antibacterial activity against Gram-negative becteria, in particular *P. aeruginosa*. Furthermore these compounds have shown exceptional β-lactamase stability, this being related to the planarity of the substituent and to the p*K*a of the catechol moiety. In the marmosets, several of these compounds also possess outstanding pharmacokinetics (equivalent or superior to ceftriaxone) which seemed highly dependent on the p*K*a of the hydroxy groups.

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