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Synthesis and Biological Activity of Some 3-Acryloxymethyl Cephalosporins[1]

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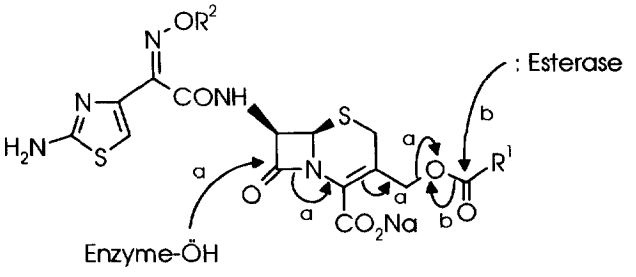
ABSTRACT: In continuation of our studies directed towards metabolically stable, 3-acyloxymethyl cephem derivatives, we report the synthesis, antibacterial activity and biological properties of **2a-f**, a series of 3-acryloxymethyl cephalosporins.

Cefotaxime **1a**, a pioneering third-generation cephalosporin, possesses high *in vitro* activity against a wide range of gram-positive and gram-negative bacterial pathogens. The mechanism of action of such 3-acetoxymethyl derivatives has been demonstrated to comprise acylative attack by the ß-lactam moiety on the penicillin-binding proteins bound to the cytoplasmic membrane of the bacterial cell-wall, with subsequent expulsion of the acetoxy nucleofuge.[2-6] *In vivo*, however, the molecule is vulnerable to esterase hydrolysis of the acetate linkage; the resulting 3-hydroxymethyl metabolite is considerably attenuated in activity.[7]



1a $R^1 = R^2 = CH_3$ (Cefotaxime) ;

1b $R^1 = CH_3$, $R^2 = H$

2 R^1 =  ;

$R^2 = H$ or CH_3

$R^{3-5} = H$ or CH_3

arrows a : interaction with penicillin-binding proteins

arrows b : deactivation *in vivo* by esterase(s)

In a previous report, we described an extension of our investigations[8] of cephalosporins containing novel 3-substituents to establish a strategy for circumventing such esterase deactivation. This provided an activating lactonyl substituent at the 3-position which could recyclise in the event of hydrolytic cleavage;[9] the series retained the *in vitro* antibacterial potency of cefotaxime. We now describe an alternative approach, based on the reduced reactivity of α,β-unsaturated esters **2** towards nucleophilic attack by water at the enoate carbonyl

group, in comparison with their saturated counterparts.[10] This is a consequence of their electronic structure and led us to predict that the conjugated series would be less compromised by esterase hydrolysis. Furthermore, we anticipated that the provision of double bond substituents would also inhibit this cleavage sterically.

The hydroxymethyl cephem **3** was obtained by known methods from 7-aminocephalosporanic acid.[11] Attempted reactions of this compound with activated acrylates (acid chloride, anhydride, mixed methanesulphonic anhydride) gave mixtures of Δ^2- and Δ^3-cephems, though in good yield (>70%). However, under Mitsunobu conditions (**Scheme 1**) almost instantaneous reaction between **3** and various unsaturated acids occurred to give the required Δ^3-cephems **4a-f** as the major products; little of the Δ^2-cephem isomers (*cf.* **11**, **Scheme 2**) was observed. Transamidation of the 7-side chain substituent using oximinoacetic acids **8** and **9**[9] then allowed elaboration to the free acids and their sodium salts **2**a-f.

In an alternative strategy, benzhydryl 6-phenylacetamido-3-chloromethyl-ceph-3-em-4-carboxylate (**10**, known as 'G Cl H')[12] was investigated as a starting material for the preparation of these acrylate derivatives, by simple nucleophilic displacement of the halogen atom by acrylate salts in dimethyl formamide as solvent. Sodium acrylate gave a complex mixture of products, largely polymeric. Higher acrylates gave some of the desired products but contaminated with inseparable Δ^2-cephems **11**. Oxidation of these mixtures with 3-chloroperbenzoic acid to restore the Δ^3-double bond *via* the Δ^3-cephem sulphoxide **12** (*cf.* **Scheme 2**) was unsatisfactory; the Δ^2- and Δ^3-esters oxidised at sulphur at considerably different rates, leading irreversibly to partial over-oxidation to the sulphones. However, the use of phase-transfer conditions for the acrylate-halogen displacement conveniently provided the Δ^2-cephem **11** as the sole product (**Scheme 2**). This obviated the foregoing complication, permitting efficient access to the required Δ^3-cephems *via* an oxidation/reduction cycle.

The methacrylate **2**c and the regioisomeric dimethacrylates **2**a and **2**e were found to exhibit interesting *in vitro* biological activities. They showed excellent activity against gram +ve and gram -ve bacteria (except for *Pseudomonads*) (**Table**), including ß-lactamase producers. They also exhibited useful activity against *Staphylococci* and *Streptococci* possessing target site-mediated resistance. Our predictions for stability of the

Fig.1: Aqueous stability of 1a, 1b, 2a. (HPLC)

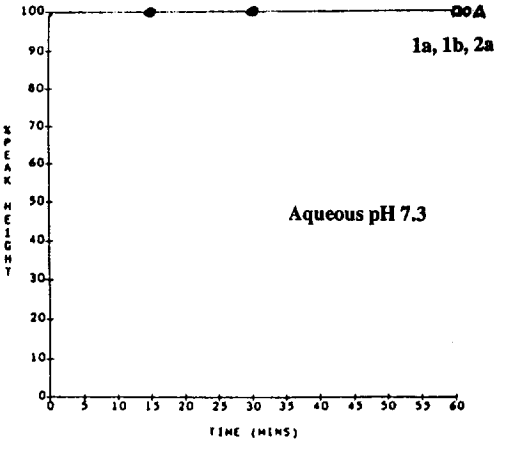
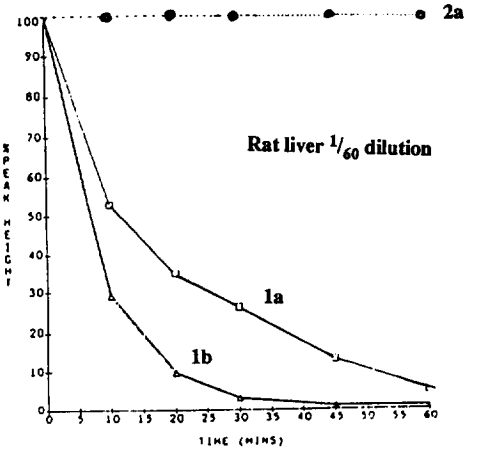
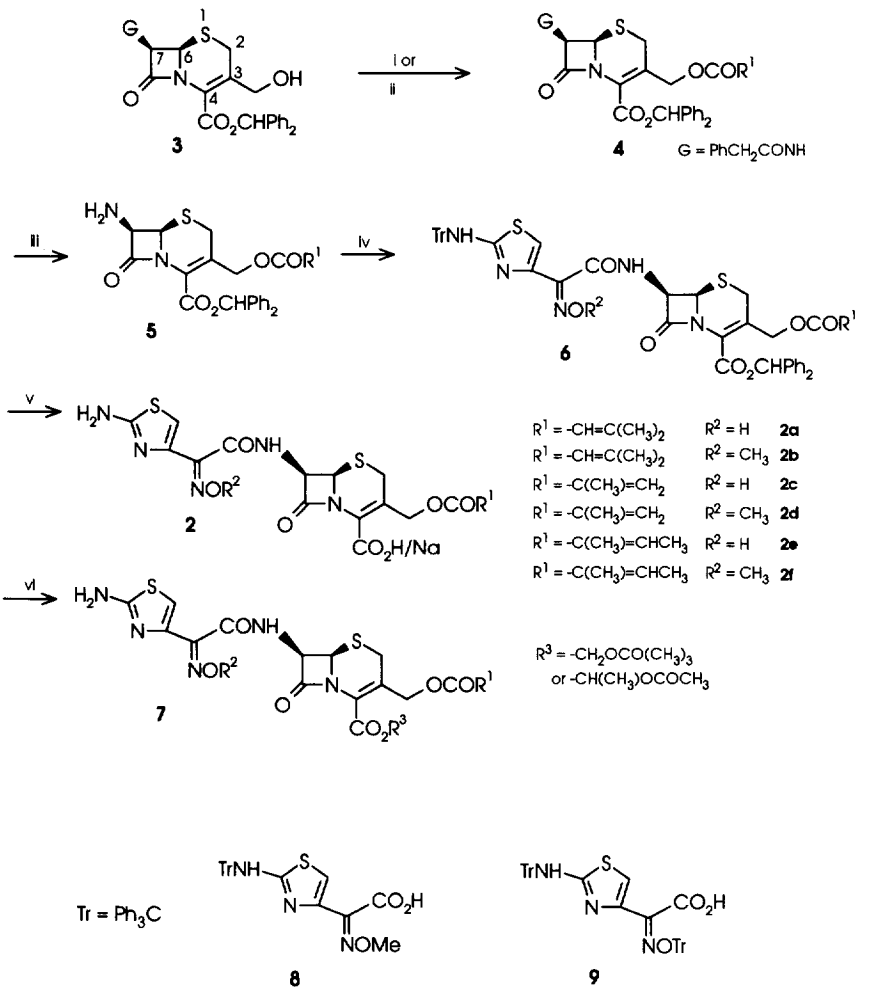


Fig.2: Stability towards Rat liver homogenate (HPLC)

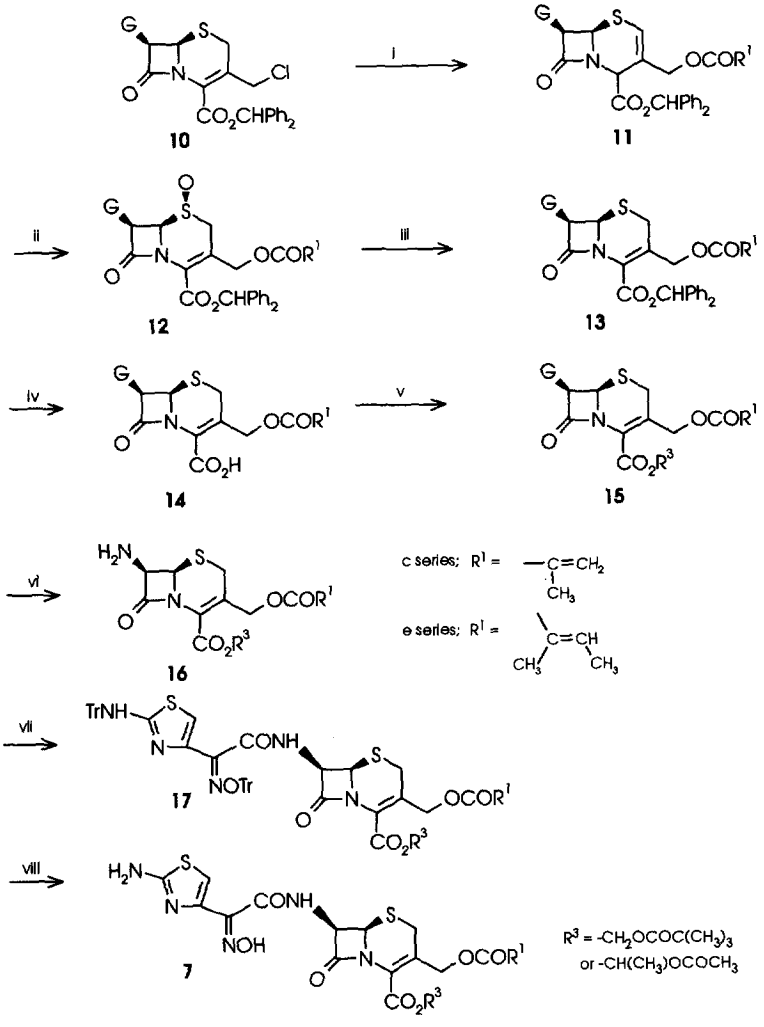


SCHEME 1



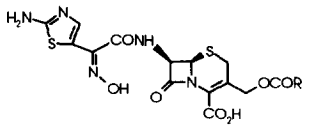
Scheme 1. *Reagents and conditions*: i, R^1COCl, pyridine; product contaminated with much **11**; ii, $EtOCON{=}NCO_2Et$ (1.2molar equiv.), Ph_3P (1.2 molar equiv.), R^1CO_2H (2.0 molar equiv.), THF, 65°C, 1min, *ca* 25% yield after chromatography; iii, 'Delft' cleavage: PCl_5, CH_2Cl_2, <0°C, N-methylmorpholine; then $MeOH/H_2O$; iv, DMF, **8** or **9**, $MeSO_2Cl$, N,N-diisopropylethylamine, -25°C.[13,14]; v, $HCO_2H/HCl/H_2O$, RT; vi, R^3 Br or R^3 I, CH_2Cl_2, H_2O, nBu_4N^+ I, pH 7, $Na_2S_2O_5$ (trace); maintained at pH 7 by addition of $Na/KHCO_3$.

SCHEME 2



Scheme 2. *Reagents and conditions*: i, R^1CO_2Na, CH_2Cl_2, H_2O, $nBu_4N^+I^-$, $Na_2S_2O_5$ (trace), pH 7; ii, 3-chloroperbenzoic acid, CH_2Cl_2; iii, DMF, PCl_3, <-25°C; iv, HCO_2H, HCl, H_2O, RT; v, R^3 X (X = Br, I) CH_2Cl_2, H_2O, $nBu_4N^+I^-$, $Na_2S_2O_5$ (trace) maintained at pH 7 by the addition of $Na/KHCO_3$; vi, 'Delft' cleavage; PCl_5, CH_2Cl_2, <0°C, N-methylmorpholine; then $MeOH/H_2O$; vii, **9**, $MeSO_2Cl$, DMF, -25°C, N,N-diisopropylethylamine; viii, HCO_2H, HCl, H_2O.

Table: Antibacterial activity MIC (μg ml^{-1}) of hydroximes **2a, 2c, 2e**

Structure	**2a**	**2e**	**2c**	**1b**	**1a**
	R= 	R= 	R= 	R= CH_3	Cefotaxime
Organism					
E.coli 10418	0.06	0.12	<0.03	0.06	<0.03
E.coli JT425†	0.5	0.5	0.12	1	0.5
E.coli ESS	<0.03	≤0.03	<0.03	<0.03	<0.03
E.coli 1077†	0.25	0.25	0.06	0.06	<0.03
K.pneumoniae T767	0.5	0.5	0.12	0.06	0.06
P.mirabilis C977	0.25	0.25	0.06	0.06	<0.03
M. morganii T361	0.25	0.12	0.06	0.06	2
H.influenzae Q1	0.06	≤0.03	<0.03	0.12	<0.03
H.influenzae NEMC†	<0.03	≤0.03	<0.03	0.06	<0.03
B.catarrhalis Ravasio†	4	4	0.50	2	0.25
P.aeruginosa 10662	>64	>64	64	>32	16
S.aureus Oxford	0.25	0.25	0.06	0.25	2
S.aureus Russell†	0.5	0.5	0.25	0.5	2
S.aureus MB9†	0.5	0.5	0.25	0.5	4
S.aureus V573*	8	8	2	>32	32
S.epidermidis PHLN20	0.25	0.25	0.06	0.25	1
S.pyogenes CN10	<0.03	≤0.03	<0.03	-	<0.03
S.agalactiae 2798	<0.03	≤0.03	<0.03	0.06	0.12
S.pneumoniae PU7*	0.5	0.5	1	2	2
S.pneumoniae 1761	<0.03	≤0.03	<0.03	0.06	<0.03
S.faecalis I	8	>64	32	4	>64

[Methoximes (**2**, $R^2 = CH_3$) in general had similar activity to hydroximes (**2**, $R^2 = H$) though less active against *Staphylococci*]

* Resistance due to modified target enzyme

† Resistance due to ß-lactamase

enoate linkage proved to be correct; for example, dimethyl acrylate **2a** retained aqueous stability and was completely stable to a rat liver homogenate preparation in which **1a** and **1b** had half-lives of the order of 0.25h or less, thus demonstrating its insusceptibility to esterases (and other degradative liver enzymes) (**Figs. 1 & 2**).

Compounds **2** (**Table**) lack pseudomonal activity necessary for parenteral usage. Nonetheless the good broad-spectrum antibacterial profiles would be suitable for an oral agent.[15] Sodium salts **2c** and **2e** showed good blood levels and long serum half-lives in mice, by the subcutaneous route, but almost no oral absorption. To enhance the oral absorption properties of these compounds their respective *in vivo* hydrolysable esters **7c** and **7e** (R^3 = pivaloyloxymethyl or acetoxyethyl) were prepared. These were derived either from the salts **2c,e** by phase-transfer alkylation (causing no Δ^2-cephem formation) or by the modified synthesis from 'G Cl H' as shown in full in **Scheme 2**. This also allowed an improved preparation of the salt forms. Administration of the esters to mice by the oral route greatly enhanced the oral bioavailability of the parent salts **7c, 7e** to *ca.* 25% of that produced on subcutaneous dosage.

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