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TRICYCLIC CEPHEMS AS INHIBITORS OF HUMAN LEUKOCYTE ELASTASE. FURO[3,4-c]CEPHAM SULFONES

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Abstract: Furo[3,4-c]cepham sulfones were efficiently obtained from 1,1-dioxo-3-methylcephem-4-ketones. Cyclization improved at one time stability of the β-lactam towards hydrolytic cleavage (pH 7.4) and reactivity (second-order inhibition rate constant k_{on}) towards human leukocyte elastase.

The chemistry of β-lactams, investigated over the last three decades in the search for compounds with improved binding to bacterial enzymes (cell-wall transpeptidases), is being re-examined with the aim of obtaining inhibitors of a different serine protease, human leukocyte elastase (HLE; EC 3.4.21.37), the defective control of which is presumably associated with several degenerative diseases.[1] The pioneering work by Merck[2,3] has shown that cephalosporin sulfones can be modified to become potent acylating agents of the serine hydroxyl at the enzyme active site. One main problem in the potential development of these compounds as drugs is their hydrolytic instability.[3] Herein we report preliminary results on tricyclic cephem sulfones, which have been addressed as a new class of hydrolytically stable HLE inhibitors.

In our previous works,[4,5] cephem-4-ketones (**1**) and derivatives functionalized at C-3' and/or C-2 were studied as more potent analogs of the 4-esters and amides discovered at Merck. One significant finding was that some of these compounds may undergo keto-enol tautomerism even in the physiological pH range, and that enolate formation boosts hydrolytic stability.[6] The resultant decrease in HLE-inhibitory activity was attributed, at least in part, to the presence of the enolate negative charge interfering with the oxyanion region of the enzyme.[3,5] To prevent this putative interaction, the synthesis of enol ethers was considered. Though alkylating agents react with **1a** at the C-2/C-4 ring positions instead of at the keto oxygen atom, intramolecular O-alkylation was readily achieved on the bromoderivative (**2a**)[5] in pH 7.4 buffer (biphasic H_2O/EtOAc system), providing the dihydrofurane (**3a**) in virtually quantitative yield (Scheme 1). Isomerization of **3a** to the novel[7] furo[3,4-c]cepham sulfone (**4a**)[8] smoothly occurred in the presence of a catalytic amount of NEt_3. Optimized conditions for the formation of **4a** (90% yield after flash chromatography) utilized plain exposure of **2a** to 1 molar equivalent of N,N-diisopropylethylamine in diluted CH_2Cl_2 solution. Analogously, the *tert*-butyl ketone (**2b**)[5] afforded **4b** in good yield (79%).

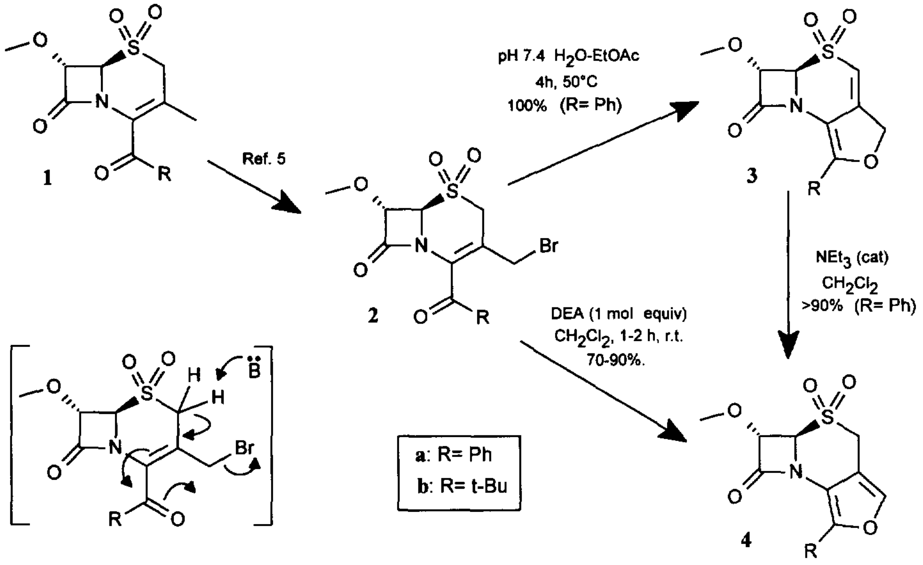
Owing to the availability[4] of 4-ketocephem sulfones mono- or di-substituted at C-2, the synthesis of the corresponding furo[3,4-c]-cepham sulfones was also considered. Since base-assisted cyclization (involving abstraction of an acidic C-2 proton) would be thwarted in the case of 2,2-disubstituted derivatives, and would proceed with epimerization on the 2-monosubstituted ones, a different approach was devised (Scheme 2). When bromides (**6a-d**), obtained from cephems (**5a-d**)[9] by radical bromination according to an established protocol,[5] were treated with silver triflate in aqueous acetone, rapid solvolysis was followed by formation of the desired furo[3,4-c]cephams (**8a-d**). Typically, reaction of **6c** for 2 hours, followed by work-up and chromatography, provided optically pure **8c** (55%), and yields of other compounds **8** ranged between 40% and 80%. When the reaction mixture from **6b** was quenched after 5 min by partitioning between EtOAc and aqueous $NaHCO_3$, pure carbinol (**7b**) was isolated in 55% yield. Simple treatment of **7b** with 1N HCl in dioxane afforded the furan (**8b**) (80%).

When tested as inhibitors of human leukocyte elastase, furo[3,4-c]cepham sulfones (**4a**) and (**4b**) proved to be superior to the corresponding 1,1-dioxo-3-methylcephem-4-ketones (**1a**) and (**1b**) both for the second-order inhibition constant, k_{on},[10] and for stability towards hydrolysis at physiological pH[11] (Table 1). The half-life for recovery of enzymic activity from the isolated adduct between HLE and **4a** was 5.6 ± 0.5 hours at 25 °C, suggesting an inhibition mechanism that involves formation of a slowly hydrolyzable ester bond between Ser-195 hydroxyl and the (cleaved) β-lactam carbonyl. Evaluation of other furo[3,4-c]cepham sulfones is in progress, and results will be reported in due time. Preliminary observations[12] show that HLE-inhibitory activity can be boosted by the introduction of leaving groups (heterocyclothio or acyloxy) at the C-2 position.

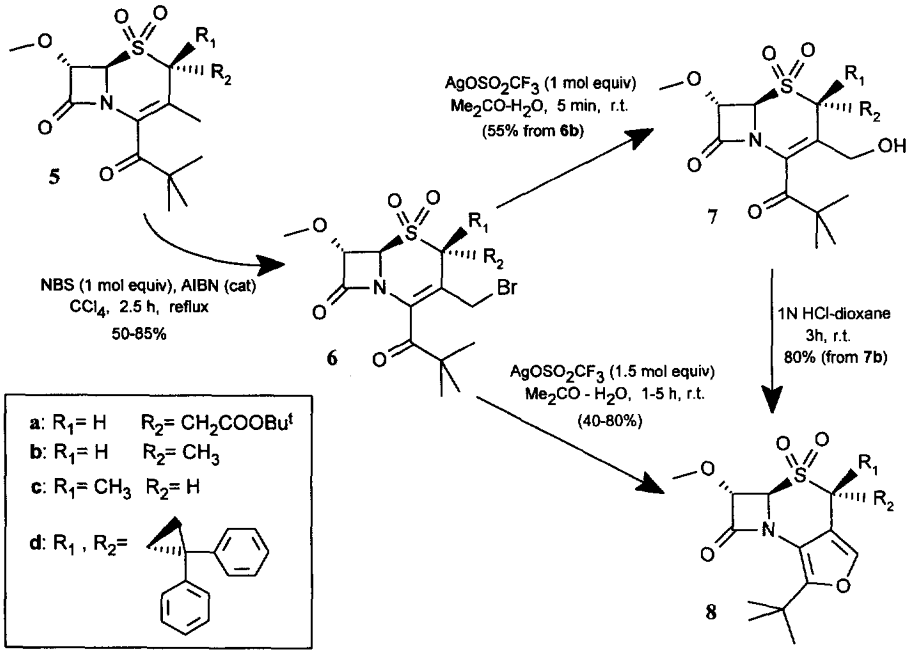
Table 1. HLE inhibitory activity (k_{on})[10] and hydrolytic stability ($t_{1/2}$)[11] of furo[3,4-c]cepham sulfones (4, 8) and their 4-ketocephem precursors (1)

COMPOUND	k_{on} ($M^{-1}sec^{-1}$) ± SD[a]	$t_{1/2}$ (hours) ± SD[a]
1a	130 ±10	26 ± 1
1b	90 ± 30	104 ± 3
4a	2000 ± 90	71 ± 1
4b	500 ± 180	180 ± 5
8a	1100 ± 150	ND[b]
8b	1000 ± 110	140 ± 3
8d	8800 ± 3300	520 ± 30[c]

a) SD = Standard deviation of parameter estimate
b) ND = Not determined
c) 20% (instead of 2%) v/v MeCN in pH 7.4 phosphate buffer



Scheme 1



Scheme 2

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(7) Previously, furo[3,4-c]cephams at the sulfide oxidation level had been prepared either from 3-acetoxymethylcephem-4-aldehydes or by total synthesis: Beeby, P.J.; Edwards, J.A. *Tetrahedron Lett.* **1976**, 3261 ; Edwards, J.A.; Guzman, A.; Johnson, R.; Beeby, P.J.; Fried, J.H. *Tetrahedron Lett.* **1974**, 2031.

(8) All new compounds gave satisfactory spectral data (^{1}H-NMR, IR, MS). See Ref. 12 for details.

(9) For the preparation of 2-alkylcephem-4-ketones (**5a-c**) see Ref. 4. Compound **5d** was prepared by addition of diphenyldiazomethane (DDM; 1 mol equiv., CH_2Cl_2, r.t. 1 h; 85%) to 4-(*tert*-butylcarbonyl)-7a-methoxy-3-methyl-2-methylene-Δ^3-cephem 1,1-dioxide.[4] A small amount (8%) of the C-2 epimer of **5d** was also isolated, the relative stereochemistry being assigned on the assumption that predominant attack of DDM occurs from the less hindered α-face: Jászberényi, J.C.; Pitlick, J.; Batta, G.; Kövér, K.E.; Kollár, K. *Magnetic Resonance in Chemistry* **1988**, *26*, 658.

(10) HLE activity (37°C, 0.055 M phosphate buffer, pH 7.4, 1% DMSO, 1% MeCN, 0.01% Triton X-100) was monitored with the fluorogenic substrate Meo-Suc-Ala-Ala-Pro-Val-7-(4-methyl)coumarylamide. Second order inhibition rate constants, k_{on} ($M^{-1}sec^{-1}$), were determined from plots of pseudo-first order inhibition rate constants against inhibitor concentration. For full details see Ref. 5.

(11) Hydrolytic stability was assayed at 37°C in 50 mM pH 7.4 phosphate buffer (2% v/v MeCN as solubilising vehicle) with initial concentration c = 0.1 mM. By monitoring the decrease of c with an automated HPLC apparatus, "chemical half-life" values, $t_{1/2}$ (hours), were obtained from plots of log(c) against time (full details in Ref. 5).

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