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

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Abstract: Thieno- and pyrrolo[3,4-c]cepham sulfones were synthesized and compared to their furo analogs for activity as human leukocyte elastase inhibitors. Pyrrolo derivatives, especially when N-alkylated, combined excellent hydrolytic stability with remarkable inhibitory activity.

In the preceding paper,[1] we described the conversion of 1,1-dioxo-3-methyl-cephem-4-ketones (**1a**, **1b**) into furo[3,4-c]cepham sulfones as part of our current research on human leukocyte elastase (HLE; EC 3.4.21.37) inhibitors. The finding that the net result of cyclization was an improvement in both inhibitory activity and hydrolytic stability prompted us to investigate related tricyclic cephems featuring bioisosteric substitution of the heteroatom.

Thieno[3,4-c]cephams were addressed through a synthetic plan involving conversion of the available bromoderivatives (**2a**, **2b**)[2,3] into the corresponding 3-mercaptomethylcephem-4-ketones (Scheme 1), in analogy to the production of furocephams from 3-hydroxymethylcephem-4-ketones[1]. Slow addition of triethylamine (in order to minimize formation of the furocepham)[1] to a solution of **2b** and thioacetic acid (1 mol equiv. each) in acetonitrile, followed by conventional work-up and flash chromatography, afforded the thioester (**3b**)[4] in over 90% yield. Under slightly different conditions (commercial potassium thioacetate in N,N-dimethylformamide), the phenyl ketone analog (**2a**) was converted to the related thioester (**3a**) (85%). Exposure of **3a**, **3b** to silver triflate (1.5 mol equiv.) in methanol overnight caused the formation of polar β-lactam derivatives, putatively the silver salts of the thiols (**A**); addition of 1N aqueous HCl to the reaction mixture gave in few minutes the desired thieno[3,4-c]cephams (**4a**, **4b**), isolated after chromatography in moderate yield. More conveniently, solvolysis of the thioesters (**3**) in a mixture of methanol and 37% hydrochloric acid (20:1) directly afforded the thienocephams (**4**) in acceptable unoptimised yields (40-60%).

Backed by our experience in constructing furocephams and thienocephams, we anticipated that a pyrrole ring fused to the cephem nucleus could be prepared by the conversion of the bromo moiety of **2a** (or **2b**) into an amino group. We planned to realize this transformation by converting the cephem bromides

into cephem azides and reducing the latter under Staudinger conditions.[5] Reaction of **2b** and silver azide[6] occurred in acetonitrile (2 h, room temperature), providing **5b** in about 90% yield; in a similar way **5a** was obtained from **2a** (Scheme 2). When triphenylphosphine (CH_2Cl_2, 2.5 h, r.t.) was added to azides (**5a, 5b**), direct cyclization to the desired pyrrolo[3,4-c]cephams (**6a, 6b**; 80-90% isolated yield) was observed. Monitoring the reaction of **5b** and PPh_3 ($CDCl_3$) by ^{1}H-NMR spectroscopy showed that no intermediate was ever present in appreciable amount, increasing quantities of **6b** being produced at the expense of **5b**; presumably, rapid formation of the pyrrole ring occurs by aza-Wittig cyclization[8,9] of iminophosphoranes (**B**).[10]

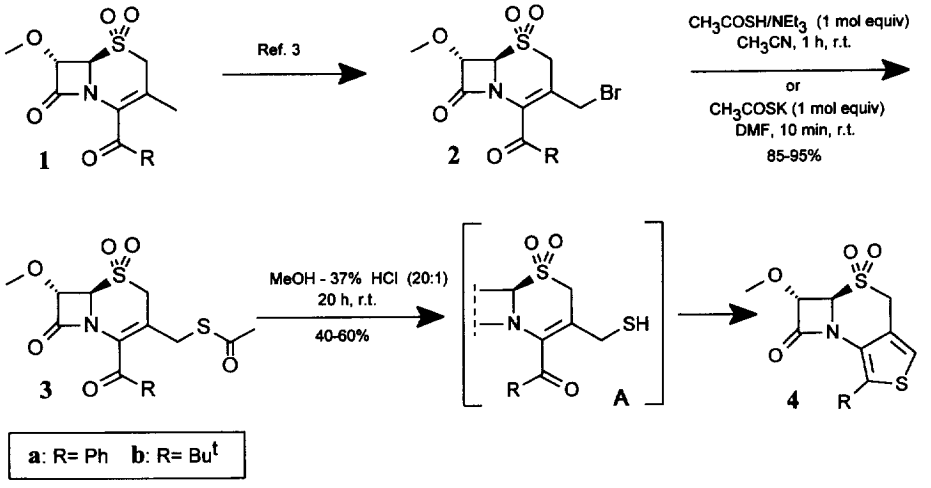
When tested in comparison with their 4-ketocephem precursors (**1a, 1b**), the thienocephams (**4a, 4b**) showed no significant increase in either HLE inhibitory activity[11] or chemical stability[12] (Table 1). On the other hand, the pyrrolo-[3,4-c]cepham sulfones (**6a, 6b**) combined fair activity with remarkable hydrolytic stability, proving to be equal or superior to the corresponding furocepham analogs.[1]

Following this result, derivatization at the nitrogen atom of **6a** was briefly examined. Somewhat harsh conditions and competing C-alkylation are often reported in the N-alkylation of pyrroles.[13] Remarkably, reaction of **6a** with activated alkyl and benzyl bromides took place very easily (Cs_2CO_3, DMF, 5-20 h); e.g., products **7a** and **8a** were obtained regioselectively in good to excellent yields. The activity gain obtained with **8a** (see Table 1), without any concurrent loss in hydrolytic stability, supports our hypothesis[1] that structural refinement of tricyclic cephem templates may provide HLE inhibitors worthy of investigation as potential drugs for the treatment of pulmonary emphysema and related ailments.

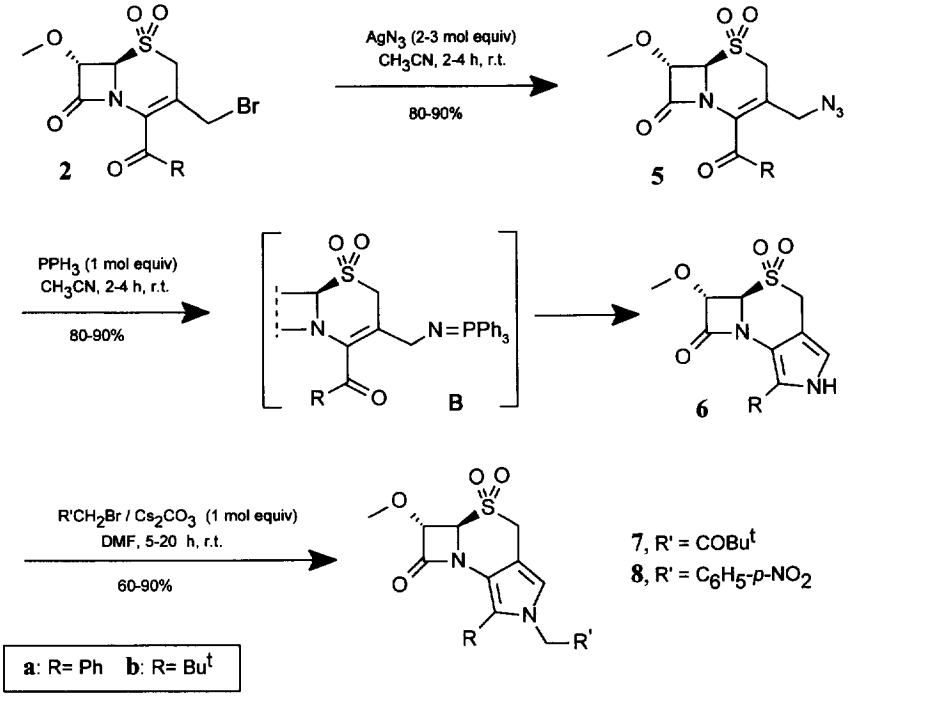
Table 1. HLE inhibitory activity (k_{on})[11] and hydrolytic stability ($t_{1/2}$)[12] of thieno[3,4-c]cepham sulfones (4) and pyrrolo[3,4-c]cepham sulfones (6, 7, 8), and their 4-ketocephem precursors (1).

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	270 ± 20	34 ± 1
4b	80 ± 10	220 ± 6
6a	2500 ± 600	570 ± 13
6b	1300 ± 150	510 ± 14
7a	2100 ± 670	800 ± 40
8a	11000 ± 2800	>3000[b]

[a] SD = Standard deviation of parameter estimate
[b] 20% (instead of 2%) v/v MeCN in pH 7.4 phosphate buffer



Scheme 1



Scheme 2

References and notes

(1) Alpegiani, M.; Bissolino, P.; Corigli, R.; Rizzo, V.; Perrone, E. *Biomed. Chem. Lett.* **1995**, *5*, preceding paper in this issue.

(2) Alpegiani, M.; Bissolino, P.; Borghi, D.; Sbraletta, P.; Tonani, R.; Perrone, E. *Heterocycles* **1993**, *36*, 1747. Alpegiani, M.; Bissolino, P.; Perrone, E.; Cassinelli, G.; Franceschi, G. *Tetrahedron Letters* **1991**, *32*, 6207.

(3) Alpegiani, M.; Bissolino, P.; Corigli, R.; Del Nero, S; Perrone, E.; Rizzo, V.; Sacchi, N.; Cassinelli, G.; Franceschi, G.; Baici, A. *J. Med. Chem.* **1994**, *37*, 4003.

(4) All new compounds gave satisfactory spectral data (^{1}H-NMR, IR, MS).
For details see: Alpegiani, M.; Bissolino, P.; Perrone, E.; Rizzo, V. *British Patent Application* No. 9310428.9 filed May 20, 1993.

(5) Staudinger conditions require treatment of azides with PPh_3 and hydrolysis of the iminophosphorane intermediates to provide amines and PPh_3O. See, for example: Vaultier, M.; Knouzi, N.; Carrié, R. *Tetrahedron Lett.* **1983**, *24*, 763. See also Ref. 8.

(6) Suspensions of silver azide in acetonitrile were prepared, according to a published procedure[7], by mixing aqueous solutions of sodium azide and silver nitrate (1 mol equiv. each), washing the precipitate by decantation with water, ethanol, diethyl ether, and eventually adding acetonitrile. CAUTION: owing to the hazard of handling schock- and heat-sensitive silver azide, experiments which involved AgN_3 were run on a millimolar scale, and care was taken not to dry the silver salt.

(7) Paul, B.; Korytnyk, W. *Carbohydrate Res.* **1978**, *67*, 457.

(8) Scriven, E.F.C.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297.

(9) Iino, Y.; Kobayashi, T.; Nitta, M. *Heterocycles* **1986**, *24*, 2437, and therein cited references.

(10) Since cyclization of **5b** was achieved under non-hydrolytic conditions ($CDCl_3$), involvement of a primary amine intermediate in this reaction is unlikely.

(11) 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 (full details in Ref. 3).

(12) Hydrolytic stability was assayed at 37°C in 50 mM pH 7.4 phosphate buffer (2% v/v MeCN as solubilizing 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.

(13) Sundberg, R.J. *Comprehensive Heterocyclic Chemistry*; Bird, C.W.; Cheeseman, G.W.M., Eds.; Pergamon Press: Oxford, 1984; pp. 353-359. See also Anderson, H.J.; Loader, C.E. *Heterocyclic Compounds: Pyrroles*; Jones, R.A., Ed.; John Wiley & Sons, Inc.: New York, 1990; pp. 397-497.

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