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SYNTHESIS AND EVALUATION OF DUAL ACTION CEPHALOSPORINS AS ELASTASE INHIBITORS [1]

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Abstract: *tert*-Butyl 7α-chloro- and 7α-methoxycephalosporanate 1,1-dioxides containing Aspirin and Diclofenac in their 3-acyloxymethyl moiety were synthesized. These dual action cephalosporins have been found to be potent elastase inhibitors and stimulators of NO biosynthesis in macrophages. © 1997 Elsevier Science Ltd.

Dual action cephalosporins (DAC) containing a covalently bound prodrug form of antibacterial 4-quinolone-3-carboxylic acids or antitumor agents such as Doxorubicin or Chlorodiazotate in their 3-acyloxymethyl or 3-carbamoyloxymethyl moiety shows promise as therapeutic agents.[2-5] The DAC concept is based on the known ability of cephalosporins to release carboxylic or N-substituted carbamic acids from its 3-acyloxymethyl fragment after splitting of the β-lactam ring.[6]

Our project was aimed at the further expansion of this concept to the synthesis and biological testing of anti-inflammatory DAC consisting of two covalently linked parts: *tert*-butyl 7α-chloro or 7α-methoxycephalosporanate dioxides which are known inhibitors of human leukocyte elastase [10] and Aspirin and Diclofenac, the well-known inhibitors of the cyclo-oxygenase involved in prostaglandin biosynthesis.[11] It was hoped that the drug combination would result in a more specific and pronounced antiinflammatory effect than the separate components.

This communication reports the synthesis and the initial pharmacological evaluation of C-3 substituted cephem sulfones **9-12**. The target compounds were prepared starting from 7-ADCA (Scheme 1) employing known and novel esterification procedures.[7-9] They were isolated in a pure 7α-stereoisomeric form and characterized by spectroscopic and elemental analysis. The only exeption was the 7-methoxy cephalosporanate sulfone **12** which was obtained as an inseparable mixture of 7α- and 7β- isomers in a ratio of 3:1.

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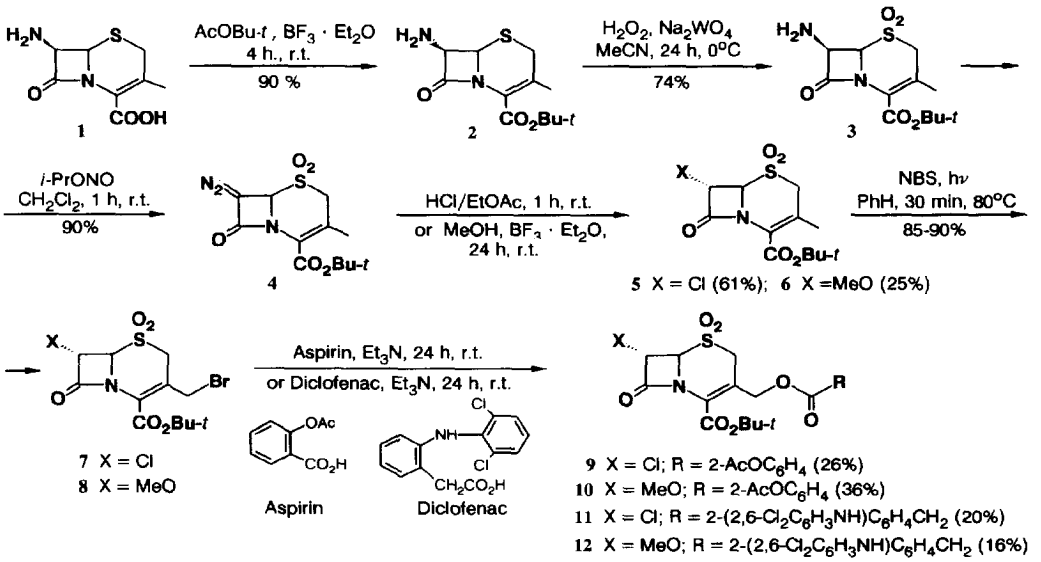

Scheme 1.

The rate of hydrolytic splitting of the β-lactam ring in DACs at pH 7.3 serves as an adequate model of the liberation of carboxylic acid from their 3-acyloxymethyl moiety.[12] The degradation of the tested compounds at 37°C monitored by HPLC led to the decrease of the parent peak and to the appearance of new peak corresponding to those of authentic sample of Aspirin or Diclofenac.[13] These measurements allowed to determine chemical half-lives for **9-12** (see Table 1) and proved the universal character of the hydrolytic splitting for different structural types of DAC.

The inhibitory properties of the potential antiinflammatory cephalosporins were determined investigating the changes in amidolytic activity of porcine pancreatic elastase (Type III) using a standard tetrapeptide *para*-nitroanilide as substrate.[14]

The data in Table 1 show that the value of the inhibitory effect directly depends on the structure of the substituent in the 3-position. In the contrast to 3-methyl unsubstituted cephalosporins **5** and **6**, DAC **9-12**, containing a leaving group, are characterized by two orders of magnitude greater inhibitory action compared to that of the *tert*-butyl 3-acetoxymethyl-7α-chlorocephalosporanate sulfone.[10]

The dependence of a steady state velocity on the inhibitor concentration was determined graphically with Lineweaver-Burk's and Dixon's plots.[15] Examples of classic noncompetitive inhibition mechanism patterns were obtained for deacetoxycephalosporins **5** and **6**. In the case of DAC **10** and **12** the inhibition occurred according to the noncompetitive mechanism. Mixed noncompetitive mechanism was observed for **11**. The analysis of the possible involvement of leaving molecules in this process determined that unlike Aspirin not altering elastase catalytic properties, Diclofenac activated this enzyme.

Table 1. Hydrolytic stability and elastase inhibitory activity of *tert*-butyl cephalosporanate sulfones

Compound	$t_{1/2}$ (hours)	IC_{50} (μM)	
		Dixon Plot	Lineweaver-Burk Plot
5		11.0±0.9	35.0±1.1
6		11.0±0.2	73.0±1.5
9	2,3	0.35±0.03	0.25±0.03
10	19		0.11±0.01
11	73	0.68±0.05	0.18±0.2
12	60		0.11±0.04
tert-butyl 3-acetoxymetyl-7α-chloroceph-3-em-4-carboxylate 1,1-dioxide [10]		0.16±0.02	0.16±0.02

It is known that activated macrophages secrete NO, regulating the course of the inflammation process.[16] Its activation by DAC in combination with the inhibition of proteases could cause a favorable therapeutic effect on the treatment of acute and chronic inflammations.

The influence of two DAC on monocultures cells of macrophages was investigated.[17] Addition of **9** and **11** in 1-10 μg/ml concentrations to the activated macrophage cells intensified their NO generation by 2.5-3 times. In the case of nonactivated cells this effect is also observed, though in not so pronounced form. These results indicate the possibility of new type macrophage antiinflammatory action intensification by the synthesized compounds.

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13. Hydrolytic stability was assayed at 37°C in 5 mM pH 7.4 phosphate buffer (20-30% v/v MeCN as solubilizing vehicle) with initial concentration 0.2-1.0 mg/ml.
14. The inhibiting effect was determined according to a standard method adapted on 96 hollow panel. Optical density was measured with a horizontal spectrophotometer Tetertek Multiscan MCC/340. Total volume 180 µl in each hollow consisted from: (a) 150 µl N-Methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide solution in concentrations of 2.0, 1.0, 0.5 and 0.25 mM dissolved in 0.1 mM Hepes (pH 7.5) and 10% DMSO; (b) 5 µg of the Porcine Pancreas Elastase (Type III) (SIGMA) dissolved in 10 µl 0.1 M phosphate buffer pH 7.4; (c) 20 µl of the DMSO solution containing inhibitors **5** and **6** in 0.4, 1.2, 3.7 and $11 \cdot 10^{-5}$ M concentrations, inhibitors **9-12** in 0.8, 2.5 and $7.4 \cdot 10^{-7}$ M concentrations and Diclofenac in 2.2, 6.7 and $20.0 \cdot 10^{-7}$ M concentrations.
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17. Cells of RAW 264.7 macrophages (obtained from ECACC) were cultivated in DMEM standard medium without an indicator and antibiotics. After the ampoule was defreezed the cells were cultivated for three days and then removed from the carrier. A part of cells in a $1 \cdot 10^{6}$ cells/ml concentration was activated in the presence and absence of tested cephalosporins with 35 µg/ml LSP (lipopolysaccharide from E.coli) and 5 units/ml of recombinant murine INFγ (interferon γ). The activation was carried out in bottles. Cells were added to the medium with the tested compounds and then activators were introduced. Activated and nonactivated cells were placed into the panel hollows. Nonactivated macrophages and macrophages treated only with cephalosporins served as a control. Cell survival (more than 90%) in the process of the experiment was determined by the number of alive cells in colorimetric test with Neutral Red (NR).[18] Registrations were made 18 hours after activation was started. Concentration of NO was determined as NO_2 concentration in panel hollows according to Greyss.[19]
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