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SYNTHESIS OF 7α-METHOXY-2-(1,3-DITHIOLAN-2-YLIDENE)CEPHEM SULPHONES. A NEW SERIES OF HUMAN LEUKOCYTE ELASTASE INHIBITORS

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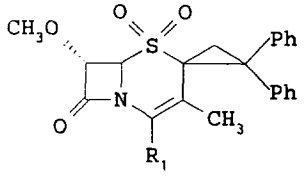
Abstract: Treatment of 3-methyl-3-cephem sulphone with sodium hydride followed by carbon disulphide and alkyl halide provides an entry to 2-(1,3-diothiolan-2-ylidene)-cephem derivatives, which are new potent inhibitors of HSE. Copyright © 1996 Elsevier Science Ltd

In 1986, Doherty et. al[1] reported that cephalosporin antibiotics can be modified to elicit potent inhibitory activity against human leukocyte elastase (HLE), a serine protease which is released from polymorphonuclear leukocytes (PMN) upon inflammatory stimuli and has been implicated as a pathogenic agent in a number of disease states such as pulmonary emphysema,[2] rheumatoid arthritis,[3] adult respiratory distress syndrome,[4] and cystic fibrosis.[5]

In recent years, much attention has focussed on the chemical modification of the C-2, C-3, C-4, and C-7 position of the cephalosporin moiety[6] in the aim of obtaining potent elastase inhibitors. Our effort in this area has produced a series of 2-spirocyclopropyl cephem sulphones (**1**) with the derivatization of the carboxylic function at the C-4 position of the cephem nucleus as esters,[7] amides,[8] and ketones,[9] and were found to elicit potent inhibitory activity against HLE.

It is further evidenced from the subsequent literature[10] that the introduction of a substituent at C-2 position of the cephem skeleton will generally increase inhibitory activity against HLE; examples of such inhibitors include 2α- and 2β-CH_3, 2α-OCH_3 and 2α-CH_2SPh. Alpegiani et.al.[6b,6c] further reported a new series of novel cephem-4-ketones (**2**) as potent elastase inhibitors.



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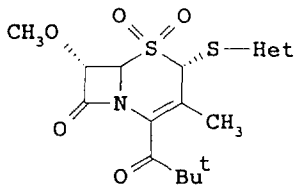

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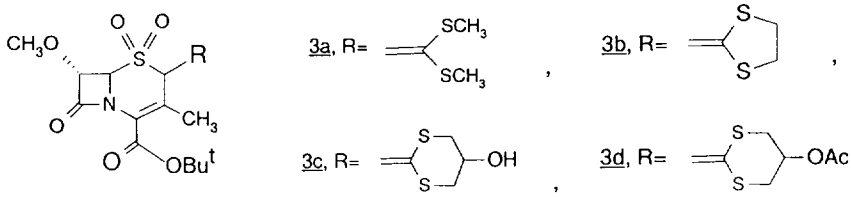
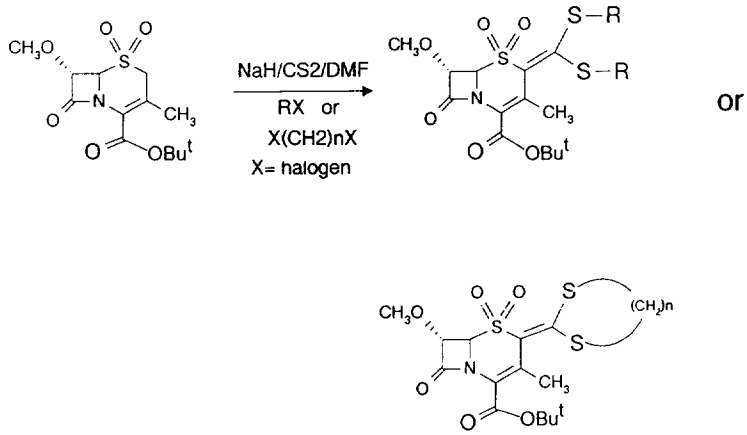
On these grounds, a research programme devoted to the synthesis and evaluation of new C-2 substituted cephem sulphones was undertaken in our laboratory. We wish to report here the synthesis and preliminary biological results obtained in this class of cephalosporin sulphones **3** (a-d).



The starting material for the preparation of the target molecules is tert-butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide (**4**) which was prepared from 7-ADCA in four steps based on the procedure described by Blacklock et.al.[11] Compound **4** on treatment with NaH in DMF followed by CS_2 and methyl iodide, gave the product **3a** in about 48% yield (Scheme). Similarly the compound **3b** was prepared in about 72% yield by treating compound **4** with NaH/DMF, followed by CS_2 and 1,2-dibromoethane. Compounds **3c** and **3d** were prepared in a similar manner by using the corresponding 1,3-dibromo derivatives. All the newly prepared compounds were tested for their elastase inhibitory activity against human sputum elastase (HSE) and



their values were compared with the starting cephem (**4**). These data (Table 2) indicate that introduction of 1,3-dithiolan-2-ylidene moiety at C-2 position of cephem sulphone nucleus potentiates the elastase inhibitory activity.

Human sputum elastase (Elastin Products, St. Louis) was assayed spectrophotometrically at 30°C by continuous monitoring of the release of p-nitroaniline from MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide at 410 nm. Incubation mixtures contained inhibitor in DMSO and enzyme in the buffer (0.01 M Na-K phosphate, 0.5 M NaCl, pH 7.6). After 10 min of incubation, substrate (0.35 mM) was added.

Compound **3a** was found to be the most potent inhibitor (IC_{50} = 0.74 x 10^{-7} M) in this series. Compounds **3**b-d are about 20-fold less active than compound **3a**, whereas the starting cephem sulphone (**4**) is a poor inhibitor.

Table 1. ^{1}H NMR Data of 7α-methoxy-2-(1,3-dithiolan-2-ylidene)cephem sulphones

Compound	Yield (%)	^{1}H NMR ($CDCl_3$, δ ppm)
3a	48	1.55 (s, 9H); 2.34 (s, 3H); 2.40 (s, 3H); 2.63 (s, 3H); 3.56 (s, 3H); 4.60 (d, 1H, J = 1.6 Hz); 5.09 (d, 1H, J = 1.6 Hz)
3b	72	1.54 (s, 9H); 2.41 (s, 3H); 3.41-3.52 (m, 4H); 3.55 (s, 3H); 4.69 (d, 1H, J = 1.8 Hz); 5.02 (d, 1H, J = 1.8 Hz)
3c	28*	1.54 (s, 9H); 2.10 (br, s, 1H, exchangeable with D_2O); 2.40 (s, 3H); 3.56 (s, 3H); 3.50-3.83 (m, 4H); 4.00-4.20 (m, 1H); 4.68 (br, s, 1H); 5.02 (br, s, 1H)
3d	16*	1.54 (s, 9H); 2.11 (s, 3H); 2.40 (s, 3H); 2.90-3.10 (m, 2H); 3.30-3.50 (m, 2H); 3.56 (s, 3H); 4.58 (br, t, 1H); 5.03 (br, t, 1H); 5.35-5.45 (m, 1H)

*Under the same reaction conditions, about 50% of the starting material (**4**) was recovered. No attempts were made to improve the yield. All the yields are based on a single experiment.

Table 2. IC_{50} values of 7α-methoxy-2-(1,3-dithiolan-2-ylidene)cephem sulphones 3a-d

Inhibitor	IC_{50}
4	18 x 10^{-7}M
3a	0.74 x 10^{-7}M
3b	3.8 x 10^{-7}M
3c	3.3 x 10^{-7}M
3d	3.2 x 10^{-7}M

Preparation of Compound **3a**

To a stirred solution of tert-butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide (**4**, 200 mg, 0.630 mmol) in DMF (0.63 mL) at 0°C under N_2 was added NaH (31.2 mg, 1.26 mmol) followed by CS_2 (0.315 mL, 5.237 mmol) and CH_3I (0.118 mL, 1.89 mmol). The mixture turned into a deep red solution which was stirred at ice-temperature for 10 min. The reaction mixture was diluted with toluene (15 mL) and water (7 mL) was added. The organic layer was separated out, washed with ice-cold water, dried (Na_2SO_4), and concentrated to give a dark orange foam which was purified by column chromatography over a silica gel column (elution with hexane-ethyl acetate, 4:1) to give the pure product (127 mg, 48%), For ^{1}H NMR, see Table 1.

Preparation of compound **3b**

To a stirred solution of tert-butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide (**4**, 200 mg, 0.630 mmol) in DMF (0.63 mL) at 0°C under N_2 was added NaH (31.2 mg, 1.26 mmol) followed by CS_2 (0.315 mL, 5.237 mmol) and 1,2-dibromoethane (0.081 mL, 0.945 mmol). The deep red color solution was stirred at 0°C for 10 min. The reaction mixture was diluted with toluene (15 mL) and washed successively with

water, brine, dried (Na_2SO_4), and concentrated. The deep yellow foam (250 mg) was purified over a silica gel column (elution with hexane-ethyl acetate, 3:2). The pure product (189 mg, 71.5%) was obtained as a bright yellow foam. For ^1H NMR, see Table 1.

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