Chemistry of Heterocyclic Compounds, Vol. 43, No. 12, 2007

SYNTHESIS AND CYTOTOXIC PROPERTIES OF 7α-CHLORO-3-METHYL-1,1-DIOXOCEPH-3-EMS, SUBSTITUTED WITH AMIDE OR KETO GROUP AT POSITION 4

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7α-Chloro-3-methyl-1,1-dioxoceph-3-ems with amide or keto group at position 4 have been synthesized by structural modification of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid. Screening of these compounds for cytotoxic activity revealed compounds with specific activity against cancer cells in vitro, capable of effective inhibition of the growth of sarcoma S-180 in vivo.

Keywords: amides of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid, amides of 2-(N,N-dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acids, 4-aryl-7α-chloro-3-methyl-1,1-dioxoceph-3-ems, cytotoxic activity.

We have shown previously that the intensity and selectivity of cytotoxic properties *in vitro* of esters of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid relative to cancer and normal cells depend on the structure of the substituent at position 4 [1]. In a continuation of this work we have synthesized and studied the cytotoxic activity of 7α-chloro-3-methyl-1,1-dioxoceph-3-ems with amide and keto groups in position 4 some representatives of, which according to literature data, are effective inhibitors of human leucocyte elastases [2, 3].

Amides of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid **5a-o** were obtained, starting from 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid (**1**) by conversion into the acid chloride using the Vilsmeier reagent. The latter was treated with the corresponding mono- or disubstituted amines **4a-o** without isolation (Scheme). Along with the amides of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid **5a-o,** the cephems **6a,d,f,g,l**, containing N,N-dimethylaminomethyl group at position 2, were obtained from the reaction mixture when *tert*-butylamine **4a**, benzhydrylamine **4d**, 3-pyridylmethylamine **4f,** *para*-methoxycarbonylaniline **4g**, and piperidine **4l** were used.

By analogy with the ^{1}H NMR spectra of esters of 2,N,N-dimethylaminomethylene-7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid [1], the characteristic shift of the resonance signals of the protons of the $=CN(CH_3)_2$ group in the *E*-isomers to the 3.00-3.10 ppm region and of the *Z*-isomers to the 3.30-3.35 ppm region, indicates that in the cephems **6a,d,l** the group $=CN(CH_3)_2$ is a mixture of the *E*- and *Z*-isomers, whereas in cephems **6f,g** it is predominantly in the form of the *E*-isomer.

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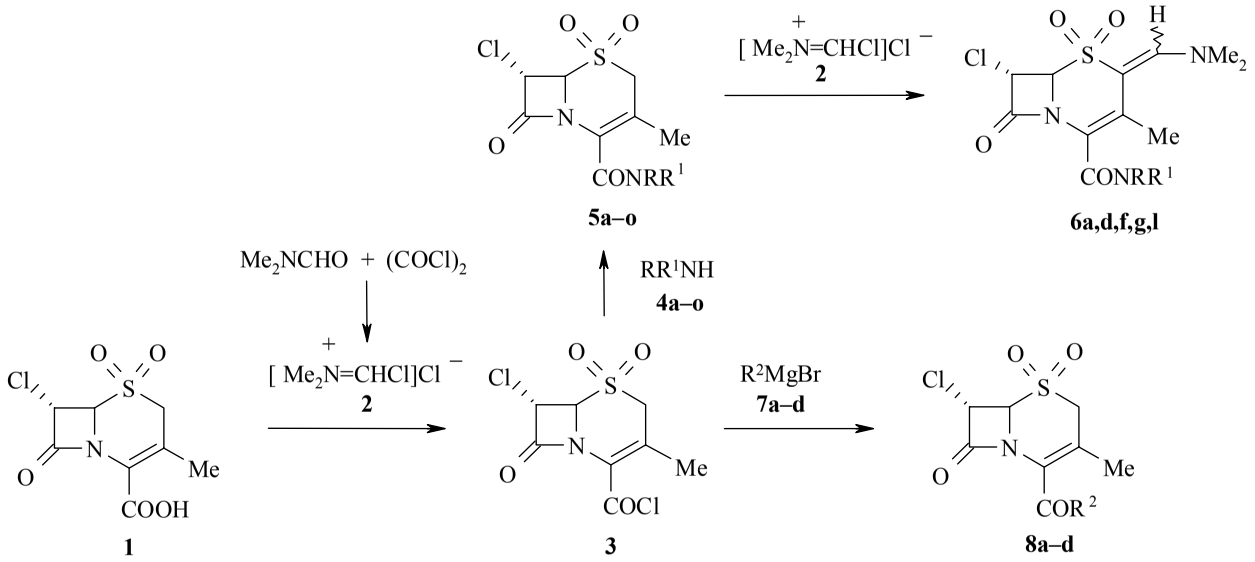
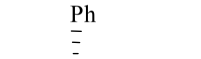
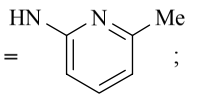
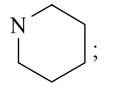
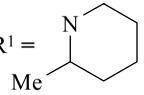
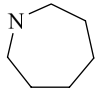
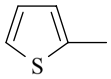
Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 12, 1849-1859, December 2007. Original article submitted October 18, 2007.



4, 5, 6 **a** NRR^1 = NHBu-*t*; **b** NRR^1 = NHOctyl-*t*; **c** NRR^1 = NHBn; **d** NRR^1 = $NHCHPh_2$; **e** NRR^1 =  ; **f** NRR^1 = $NHCH_2$Py-3; **g** NRR^1 = NHC_6H_4COOMe-4; **h** NRR^1 = NHC_6H_4CN-4; **i** NRR^1 =  ; **j** NRR^1 = NMe_2; **k** NRR^1 = NEt_2; **l** NRR^1 =  ; **m** NRR^1 =  ; **n** NRR^1 =  ; **o** NRR^1 =  ; **7, 8** **a** R^2 = *i*-Pr; **b** R^2 = *t*-Bu; **c** R^2 = Ph; **d** R^2 = 

Conversion of the 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carbonyl chloride (**3**) into the 4-oxo-substituted cephems **8a-d** using the Grignard reagents **7a-d** was carried out by a published method [3]. In addition to the previously synthesized compounds **8b** and **8c,** the new cephems **8a** and **8d** with isopropylcarbonyl and 2-thienylcarbonyl substituents at position 4 were obtained (Scheme).

The biological studies of the synthesized amides and ketones **5**, **6**, and **8** included:

a) screening for cytotoxic activity *in vitro* relative to monolayer lines of cancer cells HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma), B16 (mouse melanoma), and Neuro 2A (mouse neuroblastoma);

b) screening for cytotoxic activity *in vitro* relative to monolayer lines of normal 3T3 cells (mouse embryonic fibroplasts) and BHK (Baby Hamster Kidney – fibroplasts of golden hamster kidneys);

c) determination of the specific ability of compounds to initiate the biosynthesis of nitrogen oxide radicals in a cellular medium;

(d) calculation of the expected toxicity of the compounds tested, LD_{50} (μg/kg) [4];

(e) investigation of the anticancer activity in experiments *in vivo* in mice with grafted fast growing B16 tumor (melanoma) and slow growing S-180 tumor (sarcoma).

On the basis of the data on cytotoxic screening *in vitro* relative to cancer and normal cells, and also the calculated values of the expected toxicity, the compounds tested were separated into three groups.

Group I – compounds of low activity, the cytotoxic activity of which with respect to the tested cancer cells (LC_{50}) fell within 15 to 100 μg/kg range. The 7α-chloro-3-methyl-1,1-dioxoceph-3-ems **5b** and **5i**, the amide group of which contains *tert*-octyl- and 6-methylpyridyl groups, and also 2-N,N-dimethylamino-methylene-7α-chloro-3-methyl-1,1-dioxoceph-3-ems **6g** and **6i** with amide groups synthesized with the help of 4-methoxycarbonylaniline and piperidine, belong to this group.

Table 1. Cytotoxic Activity *in vitro* of Amides of 7α-Chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid*

Compound	Cytotoxic activity, LC_{50}, μg/ml							LD_{50}, mg/kg
	HT-1080			MG-22A			3T3	
	CV	MTT	TG_{100}	CV	MTT	TG_{100}	NR	
5a	1	2	200	2	3	250	49	597
5c	3	5	700	2	3	350	12	344
5d	0.4	0.6	500	2	2	650	21	487
6d	3	2	400	3	2	300	14	438
5e	2	2	50	2	7	100	29	537
5k	6	12	500	2	2	250	30	481
5l	3	3	15	0.4	0.2	100	40	559
5m	15	12	100	9	7	150	27	486
5n	6	7	650	2	1	500	15	373

* Here and later: LC_{50} is the concentration causing destruction of 50% of the cells; CV – coloring by crystal violet; MTT – coloring by 3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide; TG_{100} – specific NO generating activity of the compound; NR – coloring by neutral red.

Group II (Table 1) – cephems **5a,c-e,k-n, 6d –** synthesized using *tert*-butylamine, benzylamine, benzhydrylamine, methyl *R*-phenylglycine, diethylamine, piperidine, 2-methylpiperidine, and hexamethyleneimine, show high cytotoxicity both with respect to HT-1080 and MG-22A cancer cells, and normal 3T3 cells and, consequently show characteristically low values of LD_{50} (344-597 μg/kg).

Table 2. Cytotoxic Activity *in vitro* of 7α-Chloro-3-methyl-1,1-dioxocephems with Amide or Keto Group in Position 4

Compound	Cytotoxic activity, LC_{50} (μg/kg)															LD_{50}, mg/kg
	HT-1080			MG-22A			B16			Neuro2A			BHK-21		3T3	
	CV	MTT	TG_{100}	CV	MTT	TG_{100}	CV	MTT	TG_{100}	CV	MTT	TG_{100}	CV	MTT	NR	
6a	6	6	200	3	7	250	26	18	640	5	5	64	33	32	179	1146
6f	17	18	450	9	19	700	110	124	124	13	1.1	36	—*	—*	157	1138
5g	7	15	250	2	1	100	10	25	100	57	100	7	21	33	69	786
5h	8	3	500	1	0.2	200	10	25	33	45	100	33	21	29	63	718
5j	2	2	250	11	7	300	6	1	56	10	10	15	10	10	105	790
5o	28	9	260	0.4	0.6	150	20	30	100	>100	>100	2	>100	>100	316	1376
8a	2	3	450	3	3	600	39	26	50	0.8	2	113	3	4	72	671
8b	3	5	200	3	3	200	19	6	350	1	9	100	34	28	277	1235
8c	14	35	300	4	9	250	18	12	100	13	35	69	—*	—*	65	854
8d	6	10	500	4	4	650	25	29	131	2	10	850	37	47	287	1313

* Not tested.

Table 3. Therapeutic Activity *in vivo* of 7α-Chloro-3-methyl-1,1-dioxo-4-(thienylcarbonyl)ceph-3-em (**8d**)

Schedule of injection on days	Daily dose, mg/kg	Total dose, mg	Slowing of growth of the tumor, GI%			
			S-180		B-16	
			9 days	11 days	9 days	11 days
1, 2, 3, 4, 7, 8	10	60	83	76	51	32
1, 2, 3, 4, 7, 8	5	30	78	75	25	17

Group III (Table 2) – 2-substituted and unsubstituted 7α-chloro-3-methyl-1.1-dioxoceph-3-ems **6a,f, 5g,h,j,o**, the amide groups of which were obtained by using *tert*-butylamine, 3-pyridylmethylamine, *para*-methoxycarbonylaniline, *para*-cyanoaniline, dimethylamine, and morpholine. Their cytotoxicity with respect to all or some of the test cultures of cancer cells considerably exceeded those for normal cells BNK-21 and 3T3. The appearance of selectivity lead to a decreased toxicity of these compounds to values of LD_{50} equal to 718-1376 mg/kg.

The cytotoxic activity of the tested amides **5** and **6** is similar to that of the previously studied esters of 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid [1], coupled with their ability to generate NO free radicals in the cell medium. High cytotoxicity of compounds is accompanied, as a rule, by increased generation of NO radicals and the opposite.

All 4-oxo-substituted 7α-chloro-4-isopropylcarbonyl-3-methyl-1,1-dioxoceph-3-ems **8a-d**, are characterized by a highly selective cytotoxic effect in relation to cancer and normal cells, with a consequent low toxicity, LD_{50} 617-1313 mg/kg (Table 2). This is also indicated by data on the anticancer activity of 7α-chloro-3-methyl-1,1-dioxo4-(2-thienylcarbonyl)ceph-3-em (**8d**) *in vivo* in experiments on mice inoculated with tumors. It is seen from the data of Table 3 that in the case of slowly growing sarcoma S-180 a daily injection into the mouse of the test substance at doses of 5 and 10 mg/kg slowed the growth of the tumor by 75-76%. However the therapeutic effect of compound **8d** on the fast growing melanoma B16 was 2-4 times less at these doses.

The results of these studies indicate that conversion of carboxyl groups at position 4 of 7α-chloro-3-methyl-1,1-dioxoceph-3-ems into amide or keto group is a potential direction for the creation of anticancer substances, the mechanism of which, as a result of their structural similarity to inhibitors of leucocytic elastases of man, may be ascribed to the inhibition of specific elastases which facilitate the growth and proliferation of cancer cells [5-7].

EXPERIMENTAL

^1H and ^{13}C NMR spectra of $CDCl_3$ solutions with TMS as internal standard were recorded on Bruker WH90/DS (90 MHz for ^1H), Varian-Mercury BB (200 MHz for ^1H), and Varian-Mercury Plus (400 and 100 MHz for ^1H and ^{13}C respectively). Elemental analyses were carried out with a Carlo Erba analyzer with differences between experimental and calculated values of ±0.4%. Experiments were monitored by TLC on Merck Kieselgel plates with development with UV light. HPLC was carried out with a Du Pont Model 8800 equipped with a UV detector (λ = 254 nm) and a column (4.6×250 nm) filled with Symetry C_{18} or Ultrasphere octyl phases in acetonitrile–water or acetonitrile–0.1 N phosphate buffer (pH 2.5) (60:40) systems, rate 0.8-1.5 ml/min. Silica gel (Merck Kieselgel 0.063-0.230 mm) was used for preparative column chromatography. Material from Acros, Aldrich, and Sigma were used as experimental reagents. Optical density in the biological tests were carried out in 96 hole panels and determined with a Tetretek Multiscan MCC/340 horizontal spectrophotometer.

Preparation of Amides of 7α-Chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic Acid (5) and 2*E*-(N,N-Dimethylaminomethylene)-7*a*-chloro-3-methyl-1,1-dioxoceph-3-em-4-carboxylic acid (6) (General Method). To a stirred suspension of the sulfone of 7α-chloro-3-methylceph-3-em-4-carboxylic acid (300 mg, 1.13 mmol) in dichloromethane (20 ml) at room temperature a mixture of oxalyl chloride (434 μl, 3.39 mmol) in DMF (4 μl) was added. The mixture was warmed at 40°C over 20 min, cooled to room temperature and concentrated under reduced pressure. The residue, containing 7α-chloro-3-methylceph-3-em-4-carbonyl chloride, was dissolved in dichloromethane (15 ml). An amine **4** (2.8 mmol) was added to the solution cooled to -5°C. The reaction mixture was stirred for 30 min at room temperature and for 30 min at 40°, diluted with dichloromethane (40 ml), washed with 5% HCl (2×20 ml), and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was fractionated on a silica gel chromatographic column to give amides **5** and also their derivatives **6,** containing an N,N-dimethylaminomethylene group in position 2.

4-N-*tert*-Butylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5a) was obtained using *tert*-butylamine **4a** in the general method and isolated from fraction (eluent 1:1 ethyl acetate–hexane) with R_f 0.33. Yield 47 mg (13%); mp 100-102°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.40 (9H, s, C_4H_9); 1.95 (3H, s, 3-CH_3); 3.62 and 4.00 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.88 (1H, br. s, H-6); 5.31 (1H, d, ^3J =0.5, H-7); 6.44 (1H, br. s, NH). Found, %: C 44.98; H 5.44; N 8.80. $C_{12}H_{17}ClN_2O_4S$. Calculated, %: C 44.93; H 5.34; N 8.73.

Mixture of 2(*E*)-4-N-*tert*-butylaminocarbonyl-2-(N,N-dimethylaminomethylene)-7*a*-chloro-3-methyl-1,1-dioxoceph-3-em (*E*-6a) and 2(*Z*)-4-N-*tert*-butylaminocarbonyl-2-(N,N-dimethylaminomethylene)-3-methyl-1,1-dioxo-7*a*-chloroceph-3-em (*Z*-6a) (4:1) was obtained by using *tert*-butylamine in the general method and isolated from fraction (eluent 1:1 ethyl acetate–hexane) with R_f 0.18. Yield 64 mg (15%). ^{1}H NMR spectrum (400 MHz), δ, ppm (*J*, Hz): ***E*-6a** – 1.41 (9H, s, C_4H_9); 2.08 (3H, s, 3-CH_3); 3.05 (6H, s, $N(CH_3)_2$); 4.77 (1H, d, ^3J = 2, H-6); 5.08 (1H, d, ^3J = 2, H-7); 6.15 (1H, br. s, NH); 7.21 (1H, s, =$CHNMe_2$). ^{13}C NMR spectrum, δ, ppm: 21.40 (CH_3); 28.46 ($C(\underline{C}H_3)_2$); 43.32 ($(N(CH_3)_2$); 52.10 ($\underline{C}Me_3$); 55.63 (C-7); 74.39 (C-6); 98.51 (C-2); 126.42 (C-4); 131.11 (C-3); 148.46 ($\underline{C}HNMe_2$); 160.91 (CONH); 162.75 (C-8). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): ***Z*-6a** – 1.41 (9H, s, C_4H_9); 2.22 (3H, s, 3-CH_3); 3.29 (6H, s, $N(CH_3)_2$); 4.55 (1H, br. s, H-6); 5.25 (1H, br. s, H-7); 6.88 (1H, br. s, NH); 6.89 (1H, s, =$\underline{C}HNMe_2$). ^{13}C NMR spectrum, δ, ppm: 17.86 (CH_3); 28.46 ($C(\underline{C}H_3)_3$); 46.67 ($N(CH_3)_2$); 52.09 ($\underline{C}Me_3$); 55.60 (C-7); 71.09 (C-6); 99.74 (C-2); 117.82 (C-4); 124.74 (C-3); 149.34 ($\underline{C}HNMe_2$); 159.48 (C-8); 161.49 (CONH). Found, %: C 48.05; H 6.11; N 11.22. $C_{15}H_{22}ClN_3O_4S$. Calculated, %: C 47.93; H 5.90; N 11.18.

4-N-(1,1,3,3-Tetramethylbutyl)aminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5b) was obtained by use of *tert*-octylamine in the general method and isolated from fraction (eluent 1:1 ethyl acetate–hexane) with R_f 0.47. Yield 255 mg (60%); mp 102-104°C. ^{1}H NMR spectrum (90 MHz); δ, ppm (*J*, Hz): 1.00 (9H, s, 3-CH_3 octyl); 1.46 (6H, s, 2-CH_3 octyl); 1.77 (2H, d, ^2J = 6, CH_2 octyl); 2.02 (3H, s, 3-CH_3); 3.57 and 3.86 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.71 (1H, br. s, H-6); 5.26 (1H, d, ^3J = 2, H-7); 6.42 (1H, br. s, NH). Found, %: C 51.22; H 6.80; N 7.40. $C_{16}H_{25}ClN_2O_4S$. Calculated, %: C 50.99; H 6.69; N 7.43.

4-N-Benzylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5c) was obtained from benzylamine by the general method and isolated from ractions (eluent 1:1 ethyl acetate–hexane) with R_f = 0.08. Yield 100 mg (25%); mp 71-73°C. ^{1}H NMR spectrum (90 MHz); δ, ppm (*J*, Hz): 2.00 (3H, s, 3-CH_3); 3.63 and 3.89 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.51 (2H, d, ^3J = 6, CH_2 benzyl); 4.73 (1H, br. s, H-6); 5.22 (1H, d, ^3J = 2, H-7); 7.06 (1H, br. s, NH); 7.33 (5H, s, C_6H_5). Found, %: C 50.89; H 4.31; N 7.95. $C_{15}H_{15}ClN_2O_4S$. Calculated, %: C 50.78; H 4.26; N 7.90.

4-N-Benzhydrylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5d) was obtained by using benzhydrylamine **4d** in the general method and was isolated from fractions (eluent 1:3 ethyl acetate–hexane) with R_f 0.43. Yield 195 mg (40%); mp 152-154°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.93 (3H, s, 3-CH_3); 3.33 and 3.88 (2H, two d, AB system, ^2J =18, SO_2CH_2); 4.73 (1H, br. s, H-6); 5.28 (1H, d, ^3J = 2, H-7); 6.28 (1H, d, ^3J =8, $CHPh_2$); 7.08-7.60 (11H, m, $2C_6H_5$, NH). Found, %: C 58.72; H 4.63; N 6.57. $C_{21}H_{19}ClN_2O_4S$. Calculated, %: C 58.53; H 4.44; N 6.50.

A mixture of 2(*E*)-4-N-benzhydrylaminocarbonyl-2-(N,N-dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxoceph-3-em (*E*-6d) and 2(*Z*)-4-N-benzhydrylaminocarbonyl-2-(N,N-dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxoceph-3-em (*Z*-6d) (2:1) was obtained using benzhydrylamine **4d** in the general method and isolated from fractions (eluent1:1 ethyl acetate–hexane) with R_f 0.18. Yield 71 mg (13%). ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): ***E*-6d** – 2.26 (3H, s, 3-CH_3); 3.04 (6H, s, $N(CH_3)_2$); 4.77 (1H, d, ^3J =1, H-6); 5.12 (1H, d, ^3J = 8, H-7); 6.26 (1H, d, ^3J = 8, $CHPh_2$); 6.80-7.66 (12H, m, $2C_6H_5$, NH, =$CHNMe_2$). ***Z*-6d** – 2.31 (3H, s, 3-CH_3); 3.33 (6H, s, $N(CH_3)_2$); 4.55 (1H, br. s, H-6); 5.26 (1H, br. s, H-7); 6.26 (1H, d, ^3J =8, $CHPh_2$); 6.80-7.66 (12H, m, $2C_6H_5$, NH, =$CHNMe_2$). Found, %: C 59.55; H 5.09; N 8.71. $C_{24}H_{24}ClN_3O_4S$. Calculated, %: 59.31; H 4.98; N 8.65.

4-N-(1(*R*-Methoxycarbonyl)benzylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5e) was obtained from methyl *R*-phenylglycine in the general method and isolated from a fraction (eluent 1:1 ethyl acetate–hexane) with R_f 0.34. An amorphous substance, containing 98% of the basic material according to HPLC, yield 28 mg (6%). ^{1}NMR spectrum (200 MHz); δ, ppm (*J*, Hz): 2.04 (3H, s, 3-CH_3); 3.63 and 3.90 (2H, two d, AB system, ^3J = 18, SO_2CH_2); 3.75 (3H, s, OCH_3); 4.77 (1H, br. s, H-6); 5.29 (1H, d, ^3J = 2, H-7); 5.63 (1H, d, ^3J = 7, CHPh); 7.38 (5H, s, C_6H_5); 7.60 (1H, d, ^3J = 7, NH).

4-N-(3-Pyridylmethyl)aminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5f) was obtained using 3-pyridylmethylamine in the general method and isolated from fractions (eluent 3:1 ethyl acetate–hexane) with R_f 0.32. An amorphous substance, containing 98% of the basic material according to HPLC, yield 36 mg (9%). ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 2.02 (3H, s, 3-CH_3); 3.60 and 3.95 (2H, two d, AB system, ^2J = 19, SO_2CH_2); 4.55 (2H, d, ^3J = 6, CH_2Py); 4.77 (1H, br. s, H-6); 5.29 (1H, br. s, H-7); 7.26-7.42 (1H, m, H-5 Py); 7.51-7.80 (2H, m, NH, H-4 Py); 8.37-8.66 (2H, H-2,6 Py). Found, %: C 47.39; H 4.06; N 11.95. $C_{14}H_{14}ClN_3O_4S$. Calculated, %: C 47.26; H 3.97; N 11.81.

2(*E*)-(N,N-Dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxoceph-3-em (*E*-6f) was obtained by using 3-pyridylmethylamine in the general method and isolated from fractions (eluent (1:1 CH_2Cl_2–EtOH) with R_f 0.55. An oily substance, containing 98% of the basic material according to HPLC, yield 32 mg (7%). ^{1}H NMR spectrum (90 MHz), δ ppm (*J*, Hz): 2.24 (3H, s, 3-CH_3); 3.08 (6H, s, $N(CH_3)_2$); 4.48 (2H, d, ^3J = 8, C<u>H</u>$_2$Py); 4.77 (1H, br. s, H-6); 5.06 (1H, br. s, H-7); 7.11-7.33 (2H, m, =C<u>H</u>NMe and H-5 Py); 7.51-7.80 (2H, m, NH, H-4 Py); 8.35-8.64 (2H, m, H-2,6 Py). Found, %: C 49.78; H 4.51; N 13.70. $C_{17}H_{19}ClN_4O_4S$. Calculated, %: C 49.69; H 4.66; N 13.64.

4-N-(4-Methoxycarbonylphenyl)aminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5g) was obtained by using 4-methoxycarbonylaniline **4g** in the general method and isolated from fractions (eluent 1:1 ethyl acetate–hexane) with R_f 0.16. Yield 63 mg (14%); mp 226-228°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 2.11 (3H, s, 3-CH_3); 3.68 and 4.00 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 3.88 (3H, s, OCH_3); 4.80 (1H, br. s, H-6); 5.37 (1H, d, ^3J = 0.5, H-7); 7.64 and 8.04 (4H, two d, ^3J = 8, C_6H_4); 9.13 (1H, br. s, NH). Found, %: C 48.23; H 3.84; N 7.11. $C_{16}H_{15}ClN_2O_6S$. Calculated, %: C 48.19; H 3.79; N 7.02.

2(E)-(N,N-dimethylaminomethylene)-4-N-(4-methoxycarbonylphenyl)aminocarbonyl-7a-chloro-3-methyl-1,1-dioxoceph-3-em (*E*-6g) was obtained by using 4-methoxycarbonylaniline **4g** in the general method and isolated from fractions (eluent 1:1 ethyl acetate–hexane) with R_f = 0.05. Yield 31 mg (6%); mp 207-210°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 2.60 (3H, s, 3-CH_3); 3.08 (6H, s, $N(CH_3)_2$); 3.38 (3H, s, OCH_3); 4.84 (1H, br. s, H-6); 5.18 (1H, br. s, H-7); 7.24 (1H, s, C<u>H</u>NMe_2); 7.64 and 7.97 (4H, two d, ^3J = 8, C_6H_4); 8,84 (1H, br. s, NH). Found, %: C 50.36, H 4.52, N 9.11. $C_{19}H_{20}ClN_3O_6S$. Calculated, %: C 50.28; H 4.44; N 9.26.

4-N-(4-Cyanophenyl)aminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5h) was obtained using 4-cyanoaniline in the general method and isolated from fraction (eluent 1:1 ethyl acetate–hexane) with R_f = 0.18. Yield 45 mg (11%); mp 220°C. ^{1}H NMR spectrum (90 MHz, DMSO-d_6); δ, ppm (*J*, Hz): 1.86 (3H, s, 3-CH_3); 4.26 (2H, s, SO_2CH_2); 5.53 (1H, br. s, H-6); 5.88 (1H, d, ^3J = 0.5, H-7); 7.82 (4H, br. s, C_6H_4); 11.02 (1H, br. s, NH). Found, %: C 49.25; H 3.31; N 11.55. $C_{15}H_{12}ClN_3O_4S$. Calculated, %: C 50.28; H 4.44; N 11.49.

4-N-(6-Methyl-2-pyridyl)aminocarbonyl-7α-chloro-3-methyl-1,1-dioxo-ceph-3-em (5i) was obtained by using 2-amino-6-methylpyridine in the general method and isolated from fractions (eluent 1:1 ethyl acetate–hexane) with R_f = 0.24. Oily substance, containing >97% of the basic material according to HPLC, yield 28 mg (7%). ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.98 (3H, s, 3-CH_3); 2.37 (3H, s, CH_3 at Py); 3.62 and 4.02 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.84 (1H, br. s, H-6); 5.37 (1H, d, ^3J =0.5, H-7); 6.95 (1H, d, ^3J = 8, H-5 Py); 7.62 (1H, t, ^3J = 8, H-4 Py); 8.04 (1H, d, ^3J = 8, H-3 Py); 9.70 (1H, br. s, NH).

4-N,N-Dimethylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5j) was obtained by using dimethylamine in the general method and isolated from fraction (eluent 3:1 ethyl acetate–hexane) with R_f = 0.42. Yield 46 mg (14%); mp 133°C (dec.). ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 2.16 (3H, s, 3-CH_3); 3.02 and 3.07 (6H, two s, $N(CH_3)_2$); 3.51 and 3.97 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.87 (1H, d, ^3J = 1.6, H-6); 5.31 (1H, d, ^3J = 1.6, H-7). Found, %: C 41.28, H 4.61, N 9.65. $C_{10}H_{13}ClN_2O_4S$. Calculated, %: C 41.03; H 4.48; N 9.57.

4-N,N-Diethylaminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5k) was obtained by using diethylamine in the general method and isolated from a fraction (eluent 1:1 ethyl acetate–hexane) with R_f = 0.25. Yield 94 mg (26%); mp 198-201°C (dec.). ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.08 and 1.20 (6H, two t, ^3J = 7, $N(CH_2C\underline{H}_3)_2$); 1.77 (3H, s, 3-$CH_3$); 3.17-3.55 (4H, m, $N(\underline{CH}_2CH_3)_2$); 3.48 and 4.02 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.86 (1H, br. s, H-6); 5.28 (1H, d, ^3J = 1.5, H-7). Found, %: C 45.16; H 5.48; N 8.80. $C_{12}H_{17}ClN_2O_4S$. Calculated, %: C 44.93; H 5.34; N 8.73.

7α-Chloro-3-methyl-1,1-dioxo-4-piperidinocarbonylceph-3-em (5l) was obtained by using piperidine **4l** in the general method and isolated from fractions (eluent ethyl acetate) with R_f = 0.42. Yield 45 mg (12%); mp 187-189°C. ^{1}H NMR spectrum (90MHz); δ, ppm (*J*, Hz): 1.45-1.70 (6H, m, 3-CH_2, 4-CH_2, and 5-CH_2 piperidine); 1.75 (3H, s, 3-CH_3); 3.26-3.77 (4H, m, 2-CH_2 and 6-CH_2 piperidine); 3.57 and 4.05 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.94 (1H, br. s, H-6); 5,33 (1H, d, ^3J = 1.5, H-7). Found, %: C 47.17; H 5.25; N 8.49. $C_{13}H_{17}ClN_2O_4S$. Calculated, %: C 46.92; H 5.15; N 8.42.

Mixture of 2-(*E*)-(N,N-dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxo-4-piperidinocarbonylceph-3-em (*E*-6l) and 2-(*Z*)-(N,N-dimethylaminomethylene)-7α-chloro-3-methyl-1,1-dioxo-4-piperidinocarbonylceph-3-em (*Z*-6l) (3:1) was obtained by using piperidine **4l** in the general method and isolated as fractions (eluent ethyl acetate) with R_f = 0.14. Yield 22 mg (5%). ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): ***E*-6l** – 1.34-1.70 (6H, m, 3-CH_2, 4-CH_2, and 5-CH_2 piperidine); 1.73 (3H, s, 3-CH_3); 3.04 (6H, s, $N(CH_3)_2$); 3.20-3.71 (4H, m, 2-CH_2 and 6-CH_2 piperidine); 4.73 (1H, d, ^3J = 1.5, H-6); 5.06 (1H, d, ^3J = 1.5, H-7); 7.24 (1H, s, =$CHNMe_2$). ***Z*-6l** – 1.34-1.70 (6H, m, 3-CH_2, 4-CH_2, and 5-CH_2 piperidine); 1.90 (3H, s, 3-CH_3); 3.25 (6H, s, $N(CH_3)_2$); 3.20-3.71 (4H, m, 2-CH_2 and 6-CH_2 piperidine); 4.73 (1H, br. s, H-6); 5.23 (1H, d, ^3J = 1.5, H-7); 6.78 (1H, s, =$CHNMe_2$). Found, %: C 49.66; H 5.81; N 10.78. $C_{16}H_{22}ClN_3O_4S$. Calculated, %: C 49.54; H 5.72; N 10.83.

7α-Chloro-3-methyl-1,1-dioxo-4-(2-methylpiperidino)carbonylceph-3-em (5m) was obtained by using 2-methylpiperidine in the general method and isolation from fractions (eluent 2:1 ethyl acetate–hexane) with R_f = 0.22. Yield 47 mg (12%); mp 128-130°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.04-1.33 (4H, m, 3-CH_2 and 4-CH_2 piperidine); 1.33-1.68 (5H, m, 5-CH_2, 2-CH_3 piperidine); 1.71 (3H, s, 3-CH_3); 2.62-3.26 (2H, m, 6-CH_2 piperidine); 3.44 and 4.00 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.20-4.58 (1H, m, 2-CH piperidine); 4.88 (1H, br. s, H-6); 5.24 (1H, br. s, H-7). Found, %: C 48.60; H 5.71; N 8.15. $C_{14}H_{19}ClN_2O_4S$. Calculated, %: C 48.48; H 5.52; N 8.08.

4-N-Hexamethyleneiminocarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (5n) was obtained by using hexamethyleneimine in the general method and isolation from fractions (eluent 1:1 ethyl acetate–hexane) with R_f = 0.08. Yield 153 mg, 39%; mp 179-181°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.46-2.00 (8H, m, –$(CH_2)_4$– hexamethyleneimine); 1.75 (3H, s, CH_3); 3.33-3.73 (4H, m, CH_2NCH_2, hexamethylene-imine); 3.53 and 4.02 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.91 (1H, br. s, H-6); 5.35 (1H, ^3J = 1.0, H-7). Found, %: C 48.63; H 5.59; N 8.21. $C_{14}H_{19}ClN_2O_4S$. Calculated, %: C 48.48; H 5.52; N 8.08.

7α-Chloro-3-methyl-4-morpholinocarbonyl-1,1-dioxoceph-3-em (5o) was obtained by using morpholine in the general method and isolation from fractions (eluent ethyl–acetate) with R_f = 0.08. Yield 23 mg (6%); mp 153-155°C. ^{1}H NMR spectrum, (90 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.75 (3H, s, 3-CH_3); 3.31-4.00 (8H, m, 4CH_2, morpholine); 3.80 and 4.00 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.88 (1H, br. s, H-6); 5.31 (1H, d, ^3J = 1.0, H-7). Found, %: C 43.28; H 4.70; N 8.45. $C_{12}H_{15}ClN_2O_5S$. Calculated, %: C 43.05; H 4.52; N 8.37.

Preparation of 4-Acyl-7α-chloro-3-methyl-1,1-dioxoceph-3-ems (8) (General Method). A mixture of oxalyl chloride (98 μl) and DMF (10μl) was added to a stirred suspension of the sulfone of a 7α-chloro-3-methylceph-3-em-4-carboxylic acid (300 mg, 1.13 mmol) in dichloromethane (20 ml) at room temperature. The reaction mixture was stirred at 40°C for 20 min, cooled to room temperature and concentrated under reduced pressure. The residue, containing 7α-chloro-3-methyl-1,1-dioxoceph-3-em-4-carbonyl chloride, was dissolved in THF (15 ml). CuI (215 mg, 1.13 mmol) was added to the solution. The suspension was cooled to -100°C and an ethereal solution of a Grignard reagent (10 ml) (prepared by addition of the corresponding alkyl, phenyl, or thienyl bromide (0.376 mmol) to magnesium) was added under argon over 30 min. The temperature of the reaction mixture was raised to -40°C and poured into a mixture of ice, ether, and 30% aqueous NH_4Cl solution (200 ml). The organic layer was separated, washed with 5% $NaHCO_3$ (2×20 ml) and NaCl and dried over anhydrous Na_2SO_4. The solvent was evaporate under reduced pressure. The residue was fractionated on a chromatographic column containing silica gel to obtain 4-acyl-substituted cephems **8**.

7α-Chloro-4-isopropylcarbonyl-3-methyl-1,1-dioxoceph-3-em (8a) was prepared by the use of isopropyl bromide in the general method and isolated from fractions (eluent 1:1 ethyl acetate–hexane) with R_f = 0.75. Yield 20 mg (6%); mp 160-162°C. ^{1}H NMR spectrum (90 MHz), δ, ppm (*J*, Hz): 1.40 and 1.48 (6H, two d, ^3J = 6, 2CH_3); 2.11 (3H, s, CH_3); 3.64 and 3.95 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.73 (1H, br. s, H-6); 5.01-5.32 (1H, m, $CHMe_2$); 5.26 (1H, d, ^3J = 1.5, H-7). Found, %: C 45.36; H 4.91; N 4.86. $C_{11}H_{14}ClNO_4S$. Calculated, %: C 45.28; H 4.84; N 4.80.

4-*tert*-Butylcarbonyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (8b) was prepared by using *tert*-butyl bromide by a published method [3]. Yield 45 mg (13%). ^{1}H NMR spectrum (200 MHz), δ ppm (*J*, Hz): 1.53 (9H, s, C_4H_9); 2.07 (3H, s, 3-CH_3); 3.65 and 3.88 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.71 (1H, br. s, H-6); 5.26 (1H, br. s, H-7).

4-Benzoyl-7α-chloro-3-methyl-1,1-dioxoceph-3-em (8c) was prepared by using bromobenzene by a published method [3]. Yield 26 mg (7%). ^{1}H NMR spectrum (200 MHz), δ, ppm (*J*, Hz): 1.69 (3H, s, 3-CH_3); 3.65 and 4.04 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.93 (1H, d, ^3J = 1.5, H-6); 5.34 (1H, d, ^3J = 1.5, H-7); 7.46-7.77 (3H, m, H-3, H-5, Ph); 7.87-8.05 (2H, m H-2, H-6, Ph).

7α-Chloro-3-methyl-1,1-dioxo-4-(2-thienylcarbonyl)ceph-3-em (8d) was prepared by using 2-bromothiophene in the general method and isolation from fractions (eluent 1:3 ethyl acetate–hexane) with R_f 0.08. Yield 64 mg (17%); mp 183-188°C. ^{1}H NMR spectrum (200 MHz); δ ppm (*J*, Hz): 1.80 (3H, s, 3-CH_3); 3.66 and 4.00 (2H, two d, AB system, ^2J = 18, SO_2CH_2); 4.90 (1H, d, ^3J = 1.5, H-6); 5.35 (1H, d, ^3J = 1.5, H-7); 7.15-7.22 (1H, m, H-4, thienyl); 7.71-7.84 (2H, m H-3, H-5, thienyl). Found, %: C 43.56; H 3.21; N 4.18. $C_{12}H_{10}ClNO_4S_2$. Calculated, %: C 43.44; H 3.04; N 4.22.

Determination of Cytotoxic Activity *in vitro*. The cytotoxic properties *in vitro* of the substances synthesized against the monolayer cancer cells HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), B16 (mouse melanoma), Neuro2A (mouse neuroblastoma), and normal cells 3T3 (mouse embryonic fibroblasts) and BNK (kidney fibroblasts of the golden hamster) were determined on 96 holes plastic panels using the dyestuffs CV, MTT and NR by the corresponding methodology [8, 9].

Calculation of the Expected Toxicity [4]. The values of the expected toxicity LD_{50} (mg/kg) for the compounds tested were calculated from the following equation:

$$\lg LD_{50}\ (\text{mg/kg}) = 0.435 \times \lg LC_{50}\ (\text{mmol/l}) + 0.625,$$

where LC_{50} is the concentration of the tested substance (mmol/l) causing death of 50% of the fibroblasts 3T3 with cells dyed with NR.

Generation of NO Radicals by Cells. Determination of the concentration of nitric oxide radicals in cell media by Greis' method [8] was carried out on 96 holes in plastic panels. The concentrations of NO radicals obtained in this way in culture media with surviving cells after incubation for 72 h in the presence of the test substances with a concentration 50 μg/ml in the holes with a volume of 200 μl were used to calculate the values of the specific NO generating activities of the compounds (TG_{100}):

$$TG_{100} = G\ 100/C\ (\text{nmol}/\mu\text{l})$$

where G is the concentration of NO (nmol) in the cultural medium with a volume of 200 μl with surviving cells; *C* is the percent of living cells, determined by the coloring by CV.

Determination of the Anticancer Activity *in vivo*. The therapeutic activity of a compound was determined with tumors of the sarcoma S-180 and melanoma B16. Cancer cells in a quantity of 10^6 were introduced subcutaneously into male mice (CBA/DBH 2F line). The number of mice in the experimental groups was from 3 to 6. The test substances for the group of experimental mice were introduced intraperitonally as a 2% solution in DMSO with the addition of 0.3% of agarose. The control group analogously were injected with 0.2 ml DMSO with the addition of 0.3% agarose without the test substance. The effective retardation (GI) was determined as the difference in the volume of the tumors in the control and experimental groups at 9 and 11 days after implantation of the cancer cells according to the formula:

$$GI, \% = 100(1.00 - E/V);$$

where *E* is the volume of the tumor in the experimental group of animals, and *V* is the volume of the tumor in the control group of animals.

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