# Synthesis and Antimicrobial Evaluation of Some Cephem Derivatives

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Two novel cephem derivative series were synthesized: 7-(D-α-aminophenyl-acetamido-)-3-methyl-3-cephem-4-carboxylic acid monohydrate (Cephalexin) derivatives and those of 7-amino-3-(1-methyl-1*H*-tetrazol-5-yl)-thio methyl-3-cephem-4-carboxylic acid (7-AMTCA). The antimicrobial activity of the prepared compounds was studied and compared to that of known cephalosporin antibiotics of the first generation against 12 standard strains and 189 clinical isolates of *Gram*-positive and *Gram*-negative microorganisms. The Cephalexin derivatives **4a-f** show a narrow activity spectrum and are inactive while **5c** and **5d** are more active than the Cephalexin and Cephazolin antibiotics against clinically isolated *S. aureus* and *S. epidermidis* strains.

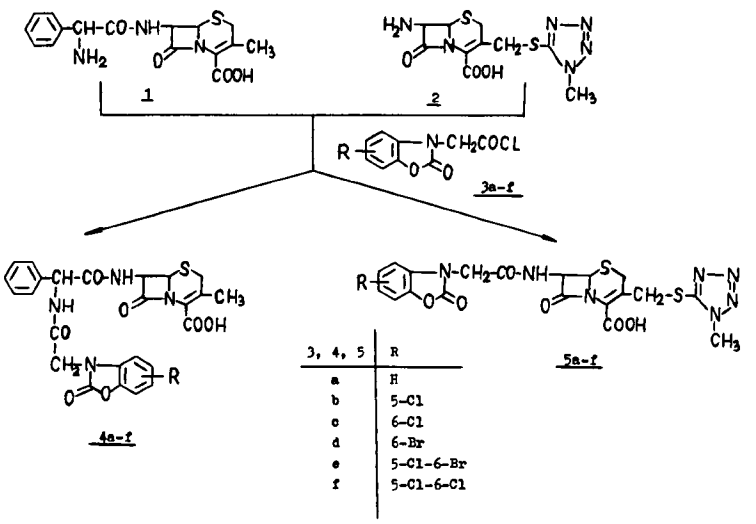
**Synthese und antimikrobielle Wirksamkeit einiger Cephemverbindungen**

Zwei neue Serien von Cephem-Derivaten, abgeleitet von 7-(D-α-aminophenyl-acetamido)-3-methyl-3-cephem-4-carbonsäure-Monohydrat (Cephalexin) bzw. Derivate der 7-Amino-3-(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl-3-cephem-4-carbonsäure (7-AMTCA) wurden synthesiert. Die antibakterielle Aktivität der neuen Verbindungen wurde gegenüber 12 Standardstämmen und 189 klinischen Isolaten *Gram*-positiver und *Gram*-negativer Mikroorganismen im Vergleich mit bekannten Cephalosporinen der ersten Generation geprüft. Die Derivate des Cephalexins **4a-f** haben ein enges Wirkungsspektrum und sind inaktiv. Die Verbindungen **5c** und **5d** übertreffen in ihrer Aktivität die Antibiotika Cephalexin und Cephazolin gegen klinisch isolierte Stämme von *S. aureus* und *S. epidermidis*.

New β-lactams of cephalosporins are frequently described. It has been established that the acyl moieties of the amino group at C-7 and the substituents at C-3 are the reasons for the extended activity spectrum of these compounds[1].

Synthesis and antimicrobial activity of 7-aminocephalosporanic acid derivatives and 7-aminodesacetoxycephalosporanic acid derivatives, containing the benzoxazolone ring, were described[2].

Acylation of the amino group in glycylcephalosporins has led to compounds with an enhanced stability against β-lactamase and an extended antibacterial spectrum[3]. The 1-methyltetrazol-5-yl-thiomethyl group in position 3 has a particularly favourable effect with respect to the antibacterial activity of numerous semisynthetic cephalosporins[3,4].




**Scheme 1**

## *Synthesis*

The extended biological activities of benzoxazolone and its derivatives stimulated us to study novel cephalosporin derivatives containing this increment using two starting cephem rings: 7-(D-α-aminophenyl-acetamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate (Cephalexin) (**1**) and 7-amino-3-(1-methyl-1*H*-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylic acid (7-AMTCA) (**2**).

Two series of cephem derivatives of the general formulas **4** and **5** were synthesized by acylation of the amino group in the side chain of the corresponding cephem structures **1** and **2**[5] (Scheme 1), using chlorides of 2-benzoxazolone-3-yl-acetic acids **3a-f**: non-substituted and substituted at C-5 or C-6, or at both positions.

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## Experimental Part

Melting points: uncorrected, Reichert-Kofler hot stage microscope.- IR-spectra (Nujol): Specord-71-IR (Zeiss), $cm^{-1}$.- $^1$H-NMR spectra: Bruker-WM 250 (250 MHz), TMS as internal standard, δ ppm.- Analytical data: Analytical Unit, Faculty of Chemistry, University of Sofia.

*General procedure for the synthesis of* **4a-f** *and* **5a-f**

Cephalexin (**1**) of 7-AMTCA (**2**) (3 mmol), after silylation of the carboxy group[5] in $CH_2Cl_2$, $Et_3N$ (3.3 mmol) and the corresponding acid chloride **3a-f** (3.3 mmol) were stirred with cooling to -5 - 0°C for 30 min, then the mixture was warmed to room temp. and stirring was continued for 2 h. After completion of the acylation (tlc control) the crude product was purified by washing with acetone and recrystallization from ethanol/$H_2O$ (1/1) or by column chromatography using $CHCl_3$-iPrOH-HCOOH 90:10:2. Analytical and spectral data: Tables 1, 2, and 3.

*Antimicrobial Screening*

*In vitro* antimicrobial activity of some representatives [**4a-f**, **5c**, and **5d**] was tested against 12 standard strains and 189 clinical isolates of *Gram*(+)

**Table 1:** Analytical and IR spectral data of new compounds **4a-f**

| Comp. No. | Yield % | M.p. °C | Molecular formula | Analysis % Calcd./Found C | H | N | S | IR cm$^{-1}$ NH | C = O |
|---|---|---|---|---|---|---|---|---|---|
| 4a | 79 | 202-203 | $C_{25}H_{22}N_4O_7S$ | 57.4 | 4.24 | 10.7 | 6.1 | 3285 | 1780;1725;1655 |
| | | | (522.5) | 56.9 | 4.51 | 10.8 | 5.6 | | |
| 4b | 86 | 225-226 | $C_{25}H_{21}ClN_4O_7S$ | 53.9 | 3.80 | 10.0 | 5.7 | 3300 | 1785;1725;1655 |
| | | | (556.9) | 54.0 | 3.77 | 9.7 | 5.6 | | |
| 4c | 74 | 217-218 | $C_{25}H_{21}ClN_4O_7S$ | 53.9 | 3.80 | 10.0 | 5.7 | 3285 | 1780;1705;1655 |
| | | | (556.9) | 54.2 | 4.04 | 9.9 | 5.2 | | |
| 4d | 76 | 219-220 | $C_{25}H_{21}BrN_4O_7S$ | 49.9 | 3.52 | 9.3 | 5.3 | 3290 | 1780;1710;1650 |
| | | | (601.4) | 49.4 | 3.78 | 9.1 | 4.9 | | |
| 4e | 81 | 255-256 | $C_{25}H_{20}ClBrN_4O_7S$ | 47.2 | 3.17 | 8.8 | 5.0 | 3290 | 1790;1720;1660 |
| | | | (635.8) | 47.4 | 3.49 | 8.5 | 4.6 | | |
| 4f | 76 | 228-229 | $C_{25}H_{20}Cl_2N_4O_7S$ | 50.7 | 3.41 | 9.4 | 5.4 | 3290 | 1785;1710;1655 |
| | | | (591.4) | 51.1 | 3.29 | 9.4 | 4.7 | | |

**Table 2:** Analytical and IR spectral data of new compounds **5a-f**

| Comp. No. | Yield % | M.p. °C | Molecular formula | Analysis % Calcd./Found C | H | N | IR, cm$^{-1}$ NH | C = O |
|---|---|---|---|---|---|---|---|---|
| 5a | 79 | 156-158 | $C_{19}H_{17}N_7O_6S_2$ | 45.3 | 3.40 | 19.4 | 3300 | 1790;1710;1695 |
| | | | (503.5) | 45.2 | 3.09 | 19.0 | | |
| 5b | 87 | 176-178 | $C_{19}H_{16}ClN_7O_6S_2$ | 42.4 | 3.00 | 18.2 | 3350 | 1785;1720;1680 |
| | | | (537.9) | 42.8 | 3.45 | 17.9 | | |
| 5c | 83 | 197-199 | $C_{19}H_{16}ClN_7O_6S_2$ | 42.4 | 3.00 | 18.2 | 3300 | 1780;1760;1710 |
| | | | (537.9) | 42.5 | 2.72 | 18.2 | | |
| 5d | 75 | 192-194 | $C_{19}H_{16}BrN_7O_6S_2$ | 39.1 | 2.77 | 16.8 | 3300 | 1780;1750;1710 |
| | | | (582.4) | 39.4 | 3.07 | 16.6 | | |
| 5e | 77 | 177-179 | $C_{19}H_{15}ClBrN_7O_6S_2$ | 37.0 | 2.45 | 15.8 | 3290 | 1780;1760;1710 |
| | | | (616.8) | 36.2 | 2.79 | 15.6 | | |
| 5f | 80 | 173-175 | $C_{19}H_{15}Cl_2N_7O_6S_2$ | 39.8 | 2.64 | 17.1 | 3290 | 1785;1755;1715 |
| | | | (572.4) | 39.5 | 3.07 | 16.8 | | |

Table 3: $^1$H-NMR data of compounds 4a-f and 5a-f

| No. | δ (ppm) , |
|---|---|
| 4a[a] | 1.99(s,3H,3-$CH_3$);3.26(d,J=18.4Hz,1H,2-$CH_2$);3.49(d,J=18.4Hz,1H,2-$CH_2$);4.65(s,2H,N-$CH_2$); 4.96(d,J=4.6Hz,1H,6-CH);5.62(dd,J=4.6 and J=8Hz,1H,7-CH); 5.66(d,J=7.8Hz,1H,11-CH); 7.14-7.48(m,9H,Ar-H);9.12(d,J=7.8Hz,1H,12-NH);9.37(d,J=8Hz,1H,9-NH) |
| 4b[a] | 2.02(s,3H,3-$CH_3$);3.23(d,J=18.6Hz,1H,2-$CH_2$);3.47(d,J=18.6Hz,1H,2-$CH_2$)4.66(s,2H,N-$CH_2$); 4.94(d,J=4.4Hz,1H,6-CH);5.59(dd,J=4.4 and J=8.8Hz,1H,7-CH);5.61(d,J=7.6Hz,1H,11-CH); 7.17-7.49(m,8H,Ar-H);9.09(d,J=7.6Hz,1H,12-NH);9.38(d,J=8.8Hz,1H,9-NH) |
| 4c[a] | 1.97(s,3H,3-$CH_3$);3.27(d,J=18.0Hz,1H,2-$CH_2$);3.41(d,J=18.0Hz,1H,2-$CH_2$);4.67(s,2H,N-$CH_2$); 4.95(d,J=4.7Hz,1H,6-CH);5.61(dd,J=4.7 and J=8.1Hz,1H,7-CH);5.70(d,J=8.2Hz,1H,11-CH); 7.24-7.57(m,8H,Ar-H);9.16(d,J=8.2Hz,1H,12-NH);9.36(d,J=8.1Hz,1H,9-NH) |
| 4d[a] | 1.99(s,3H,3-$CH_3$);3.24(d,J=18.4Hz,1H,2-$CH_2$);3.48(d,J=18.4Hz,1H,2-$CH_2$);4.66(s,2H,N-$CH_2$); 4.95(d,J=4.4Hz,1H,6-CH);5.60(dd,J=4.4 and J=8.0Hz,1H,7-CH);5.66(d,J=7.6Hz,1H,11-CH); 7.14-7.65(m,8H,Ar-H);9.13(d,J=7.6Hz,1H,12-NH);9.37(d,J=8.0Hz,1H,9-NH) |
| 4e[a] | 1.99(s,3H,3-$CH_3$);3.24(d,J=18.4Hz,1H,2-$CH_2$);3.48(d,J=18.4Hz,1H,2-$CH_2$);4.67(s,2H,N-$CH_2$); 4.96(d,J=4.8Hz,1H,6-CH);5.62(dd,J=4.8 and J=8.6Hz,1H,7-CH);5.66(d,J=7.2Hz,1H,11-CH); 7.32-7.85(m,7H,Ar-H);9.13(d,J=7.2Hz,1H,12-NH);9.37(d,J=8.6Hz,1H,9-NH) |
| 4f[a] | 2.09(s,3H,3-$CH_3$);3.21(d,J=18.5Hz,1H,2-$CH_2$);3.44(d,J=18.5Hz,1H,2-$CH_2$);4.68(s,2H,N-$CH_2$); 4.94(d,J=4.6Hz,1H,6-CH);5.60(dd,J=4.6 and J=8.3Hz,1H,7-CH);5.71(d,J=8Hz,1H,11-CH); 7.32-7.80(m,7H,Ar-H);9.18(d,J=8Hz,1H,12-NH);9.38(d,J=8.3Hz,1H,9-NH) |
| 5a[b] | 3.72(s,2H,2-$CH_2$);3.97(s,3H,N-$CH_3$);4.30(d,J=13.4Hz,1H,$CH_2$-S-Het);4.41(d,J=13.4Hz,1H, $CH_2$-S-Het);4.60(s,2H,N-$CH_2$);5.00(d,J=4.8Hz,1H,6-CH);5.73(dd,J=4.8 and J=7.8Hz,1H, 7-CH);6.96-7.29(m,4H,Ar-H);9.47(d,J=7.8Hz,1H,NH) |
| 5b[b] | 3.73(s,2H,2-$CH_2$);3.94(s,3H,N-$CH_3$);4.31(d,J=13.6Hz,1H,$CH_2$-S-Het);4.41(d,J=13.6Hz,1H, $CH_2$-S-Het);4.60(s,2H,N-$CH_2$);5.07(d,J=4.6Hz,1H,6-CH);5.68(dd,J=4.6 and J=8.2Hz,1H, 7-CH);7.17-7.43(m,3H,Ar-H);9.39(d,J=8.2Hz,1H,NH) |
| 5c[a] | 3.63(d,J=18.1Hz,1H,2-$CH_2$);3.78(d,J=18.1Hz,1H,2-$CH_2$);3.94(s,3H,N-$CH_3$);4.24(d,J=13.3Hz, 1H,$CH_2$-S-Het);4.37(d,J=13.3Hz,1H,$CH_2$-S-Het); 4.62(d,J=6.2Hz,2H,N-$CH_2$);5.08(d,J=4.6Hz, 1H ,6-CH);5.71(dd,J=4.6 and J=8.0Hz,1H ,7-CH);7.21-7.58(m,3H,Ar-H); 9.40(d,J=8.0Hz, 1H,NH) |
| 5d[b] | 3.68(d,J=18.4Hz,1H,2-$CH_2$);3.77(d,J=18.4Hz,1H,2-$CH_2$);3.97(s,3H,N-$CH_3$);4.30(d,J=13.4Hz, 1H,$CH_2$-S-Het);4.41(d,J=13.4Hz,1H,$CH_2$-S-Het);4.59(s,2H,N-$CH_2$);4.99(d,J=4.7Hz,1H,6-CH); 5.73(dd,J=4.7 and J=8.0Hz,1H,7-CH);6.88-7.37(m,3H,Ar-H);9.48(d,J=8.0Hz,1H,NH) |
| 5e[a] | 3.60(d,J=18.2Hz,1H,2-$CH_2$);3.80(d,J=18.2Hz,1H,2-$CH_2$);3.94(s,3H,N-$CH_3$);4.30(d,J=13.2Hz, 1H,$CH_2$-S-Het);4.44(d,J=13.2Hz,1H,$CH_2$-S-Het);4.63(d,J=5.2Hz,2H,N-$CH_2$);5.07(d,J=4.8Hz, 1H,6-CH);5.73(dd,J=4,8 and J=8.2Hz,1H,7-CH);7.66 and 7.92(two s, each 1H,Ar-H);9.37 (d,J=8.2Hz,1H,NH) |
| 5f[a] | 3.60(d,J=18.0Hz,1H,2-$CH_2$);3.76(d,J=18.0Hz,1H,2-$CH_2$);3.94(s,3H,N-$CH_3$);4.26(d,J=13.0Hz, 1H,$CH_2$-S-Het);4.35(d,J=13.0Hz,1H,$CH_2$-S-Het);4.63(d,J=5.1Hz,2H,N-$CH_2$);5.07(d,J=4.8Hz, 1H,6-CH);5.71(dd,J=4.8 and J=7.9Hz,1H,7-CH);7.66 and 7.82(two s , each 1H,Ar-H);9.38 (d,J=7.9Hz,1H,NH) |

a) in DMSO-$d_6$

b) in $CDCl_3$/DMSO-$d_6$

(*) In NMR descriptions s = singlet, d = doublet, dd = double doublet, m = multiplet

and *Gram*(-) microorganisms and was compared to that of known cephalosporin antibiotics of the first generation i.e. Cephalotin, Cephalexin, Cefatrexyl and Cephazolin. Minimum inhibitory concentrations (MIC) were determined by a standard reference 2-fold serial agar dilution method in *Mueller-Hinton* agar after incubation at 37°C for 20 h with an inoculum size of $10^7$ cfu/ml[2,6,7].

*Antimicrobial Evaluation*

*Antibacterial activity against standard strains*

Against *Gram*(+) microorganisms *Staphylococci* and *Streptococci* the Cephalexin derivatives 4a-f show a considerably lower activity than that of the reference antibiotics (2 to 66 times), (Table 4). Against some specific microorganisms only some of these derivatives show the same activity as that of Cephalexin or Cephazolin. Against *Gram(-) microorganisms E. coli* ATCC 25922 and *K. pneumoniae* 450 the cephems 4a-f are less active (4 to 16 times) than Cephalotin, Cefatrexyl, and Cephazolin and show doubly higher activity than that of Cephalexin.

Compounds 5c and 5d (Table 4) are very active against *Gram*(+) bacteria, *B. subtilis* being however an exception (MIC > 128 µg/ml). The antimicrobial activity of these compounds is almost the same as that of Cephalotin and Cefatrexyl and 2 to 33 times higher than that of Cephazolin and Cephalexin. The same compounds are more active also against the

Table 4: Microbiological activity of compounds 4a-f, 5c, and 5d against different bacteria

| Standard strains (*) | Minimum inhibitory concentration ( MIC ), μg/ml | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4a | 4b | 4c | 4d | 4e | 4f | 5c | 5d | Cepha-lotin | Cefat-rexyl | Cepha-lexin | Cepha-zolin |
| 1 | 2.0 | 4.0 | 4.0 | 8.0 | 4.0 | 8.0 | 0.06 | 0.12 | 0.12 | 0.12 | 2.0 | 0.25 |
| 2 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 4.0 | 0.06 | 0.06 | 0.06 | 0.06 | 1.0 | 0.25 |
| 3 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 16.0 | 0.5 | 0.5 | 0.5 | 0.25 | 0.5 | 0.25 |
| 4 | 2.0 | 1.0 | 2.0 | 2.0 | 1.0 | 2.0 | 0.06 | 0.06 | 0.03 | 0.03 | 0.5 | 1.0 |
| 5 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 16.0 | 1.0 | 1.0 | 0.5 | 0.25 | 2.0 | 2.0 |
| 6 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 4.0 | 8.0 | 8.0 | 2.0 |
| 7 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 1.0 | 0.5 | 0.5 | 1.0 | 16.0 | 2.0 |
| 8 (**) | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 1.0 | 0.5 | 0.5 | 1.0 | 16.0 | 2.0 |

(*) Abbreviations: 1: *Staphylococcus aureus* ATCC 25923, 2: *Staphylococcus epidermidis* ATCC 50, 3: *Streptococcus pyogenes* 14/58 tp. 49, 4: *Streptococcus pyogenes* 569 gr. A, 5: *Streptococcus faecalis* ATCC 8043, 6: *Bacillus subtilis* ATCC 6633, 7: *Escherichia coli* ATCC 25922, 8: *Klebsiella pneumoniae* 450

(**) Standard strains: 9: *Sarcina lutea* ATCC 9341, 10: *Pseudomonas aeruginosa* ATCC 27853, 11: *Proteus mirabilis* 56/10 and 12: *Proteus morganii* 235/12A are completely resistant (MIC > 128 μg/ml)

Table 5: Antibacterial activities of cephem derivatives 5c and 5d against clinical isolates

| Organism : ( no. of strains ) | S.aureus (80) | | | S.epidermidis (20) | | | S.agalactiae (11) | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound No. | (μg/ml) | | | (μg/ml) | | | (μg/ml) | | |
| | Range | $MIC_{50}$ | $MIC_{90}$ | Range | $MIC_{50}$ | $MIC_{90}$ | Range | $MIC_{50}$ | $MIC_{90}$ |
| 5c | 0.03- >128 | 0.17 | 1.5 | 0.03-0.12 | 0.05 | 0.10 | 2.0->128 | 5.0 | >128 |
| 5d | 0.03- >128 | 0.16 | 2.0 | 0.03-0.12 | 0.04 | 0.06 | 2.0->128 | 6.0 | >128 |
| Cephalotin | 0.06- >128 | 0.17 | 1.0 | 0.03-0.12 | 0.07 | 0.10 | 1.0->128 | 10.0 | 16 |
| Cefatrexyl | 0.03- >128 | 0.09 | 0.21 | 0.03-0.12 | 0.04 | 0.08 | 1.0->128 | 2.5 | 16 |
| Cephalexin | 0.25- >128 | 1.52 | 128 | 0.25-2.0 | 0.77 | 1.57 | 8.0->128 | 7.14 | 30.4 |
| Cephazolin | 0.12- >128 | 0.31 | 0.75 | 0.12-0.25 | 0.13 | 0.22 | 2.0->128 | 3.75 | 16 |

Table 6: Susceptibility of 189 *Gram*-positive and *Gram*-negative clinical isolates

| Organism (No. of strains) | Comulative percentage of inhibited strains, % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4a | 4b | 4c | 4d | 4e | 4f | 5c | 5d | Cepha-lotin | Cefat-rexyl | Cepha-lexin | Cepha-zolin |
| 1. S.aureus (80) | 81 | 71 | 80 | 73 | 65 | 47 | 95 | 95 | 94 | 98 | 88 | 96 |
| 2. S.epidermidis (20) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 3. S.agalactiae (11) | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 54 | 45 | 73 | 64 | 67 |
| 4. E.coli (22) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 83 | 83 | 100 | 100 |
| 5. K.pneumoniae (12) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 87 | 83 |
| 6. Enterobacter (16) (*) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 56 | 56 |

(*) *Citrobacter* (16) and *P. aeruginosa* (12) are completely resistant both to the new derivatives and to the reference antibiotics

above mentioned *Gram*(-) strains, the highest activity being observed with respect to Cephalexin, up to 32 times.

Both the new cephem derivatives and the reference antibiotics are inactive against the rest of standard strains: Nos. 9, 10, 11, and 12.

*Antibacterial activity against clinical isolates*

189 clinical *Gram*(+) and *Gram*(-) microorganisms were tested. The results obtained with *Gram*(+) strains (MIC range, $MIC_{50}$, $MIC_{90}$[8]) are

presented in Table 5 only for **5c** and **5d**, since only these compounds show an activity higher than that of the reference antibiotics. The calculated cumulative percentage of inhibited strains for all compounds studied is shown in Table 6[8].

The new compounds are particularly active against strains of *S. epidermidis*, **5c** and **5d** showing the highest activity which is equal to that of Cephalotin and Cefatrexyl and higher (2 to 26 times) than that of Cephalexin and Cephazolin. All strains are inhibited at concentrations of 0.12 µg/ml. The activity of the **4a-f** cephems is four times lower than that of Cephalexin: strain growth is suppressed in all cases at a concentration of 8 µg/ml of these derivatives.

The sensitivity-% of the 80 *S. aureus* strains studied is the highest towards **5c, 5d, 4a, and 4c**, resp. 95, 95, 81, and 80%. The derivatives **4b, 4d, 4e**, and **4f** suppress the growth of a less number of strains - from 47 to 73%, Table 6. The activity of **5c** and **5d** (Table 5) is comparable to that of Cephalotin and several times higher than that of Cephalexin. Cephalexin derivatives **4a-f** are inactive against *S. aureus* strains ($MIC_{90}$ > 128 µg/ml).

Only compounds **5c** and **5d** suppress the growth of *S. agalactiae* strains: 54%. These cephems show, however, a lower activity than that of known antibiotics used for comparison. The Cephalexin derivatives **4a-f** do not inhibit these strains, $MIC_{90}$ > 128 µg/ml.

All derivatives studied are inactive against clinical isolates of *Gram*(-) microorganisms, Table 6.

This screening shows that the compounds have a narrow spectrum of antimicrobial activity, limited to the range of *Gram*(+) cocci. Against *Gram*(-) microorganisms they are either inactive or their activity is weak.

The Cephalexin derivatives **4a-f** show a considerably lower activity than that of the reference four antibiotics against standard strains; they are inactive against clinical isolates, although the growth of a considerable % of *S. aureus* and *S. epidermidis* clinical isolates is suppressed at given concentrations of these compounds. Against standard strains compounds **5c** and **5d** have a broader activity spectrum than that of **4a-f**.

They show a higher activity against *S. aureus* and *S. epidermidis* clinically isolated strains which is comparable to that of Cephalotin and Cefatrexyl antibiotics and manyfold higher than that of Cephalexin and Cephazolin.

In Conclusion, the results of our study broaden the present knowledge of the structure-activity relationship of the cephalosporins class.

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