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3-(치환) 피로리딘세파로스포린의 합성과 항균활성평가

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Synthesis and Antimicrobial Evaluation of 3-(Substituted) Pyrrolidine Cephalosporins

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Abstract — To develop new cephalosporin antibiotics with improved antibacterial activities, a series of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[5-(heterocycle)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (**14~18**) having aminothiazol carboxymethylethoxyimino group on the C-7 position and (heterocycle) thiomethyl pyrrolidinthiomethyl group on the C-3 position of the cephem ring were synthesized. These compounds were tested for antimicrobial activity *in vitro* against Gram(+) and Gram(−) bacteria. Compounds **15** and **16** showed remarkable antibacterial activity against *Salmonella typhimurium* TV119 and *Alcalienes faecalis* KCTC1004, but most of compounds showed lower activity than cefotaxime

Keywords □ ACLE, pyrrolidine, aminothiazole-alkoxyiminocephalosporin, antibacterial activiy.

Cephalosporin항생제의 cephem핵의 7번위치에 도입시킨 aminothiazole-alkoxyimino기는 균의 외막투과성을 증가시켜 광범위 항균 spectrum을 갖게하고, β-lactamase에 대한 안정성과 penicillin binding protein (PBP)에 대한 결합친화성을 상승시켜 항균작용을 증가시킨다. Alkoxyimino부위는 methoxyimino기를 가진 ceftizoxime,[1-3] cefotaxime,[4-5] cefmenoxime,[6] 그리고 ceftriaxone[7]등이 보고되었으며 carboxymethoxyimino기와 carboxymethylethoxyimino기를 가진 cefixime[8]와 ceftazidime[9]등이 보고되었다. 3번위치에 heterocyclic-thiomethyl,[10-14] quaternary ammonium salt,[15-23] vinyl[24-29] 및 catechol[30-37]등을 도입시킨 cephalosporin계 항생제들은 항균력, 흡수, 대사를 결정짓는 중요한 부위로서 *pseudomonase*균과 같은 G(−)균에 강력한 항균력을 가지면서 β-lactamase에 대한 저항성을 향상시키는 것으로 보고되어 있다. 또한 4번위치의 carboxy기를 ester로 전환시킨 화합물들은 흡수율을 개선하고 생체이용율을 높일 목적으로 prodrug형태의 세파로스포린계[38-40] 항생제가 보고되어 있다. 본저자는 cephem ring의 7번위치에는 aminothiazole-carboxymethylethoxy기를 도입시키고, 한편 carbapenem의 2번위치에 pyrrolidinthio기가 광범위항균spectrum을 갖게하고 G(−)균과 같은 *pseudomonas*균에 대한 항균력을 증가시킨다는 보고[41-44]에따라 pyrroridine에 여러 가지 약리활성이 기대되는 복소환 유도체를 도입시킨 heterocycle-pyrrolidine유도체를 합성하여 3 번위치에 도입시킨 heterocycle-pyrrolidinecephalosporin계 항생물질을 합성하여 G(+), G(−) 및 fungus에 대하여 항균력을 실험하여 보고하고자 한다.

실 험

시약 및 기기 – 본 실험에 사용된 시약들은 Aldrich

본 논문에 관한 문의는 이 저자에게로
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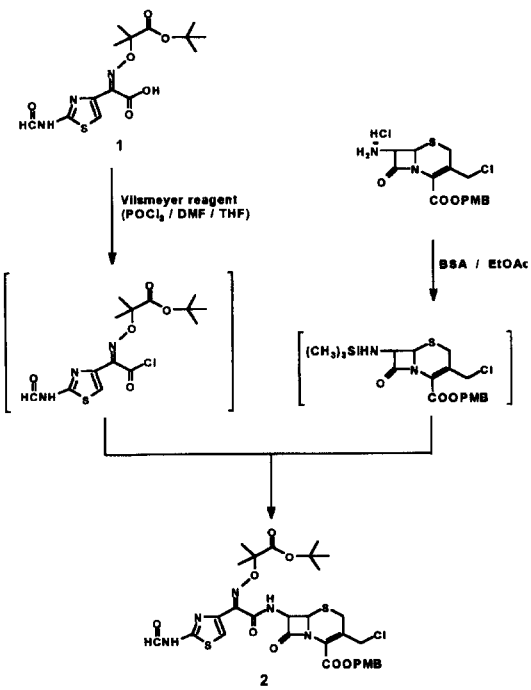
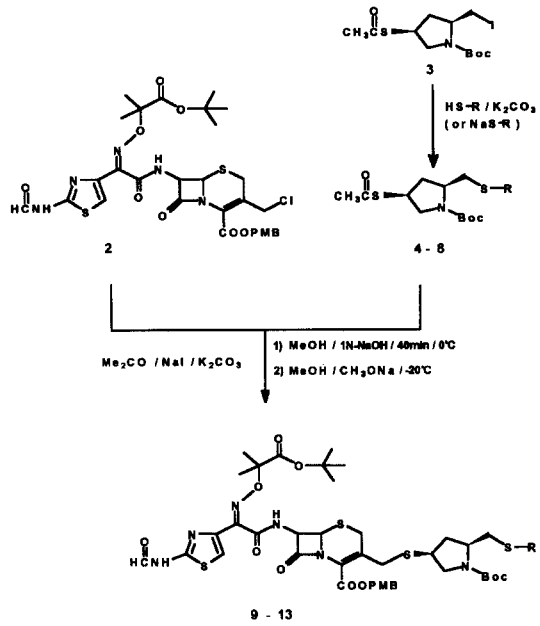
Co., Sigma Co., Tokyo Kasei., Fluka Co. 에서 구입한 특급 및 일급시약을 사용하였으며 (Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl)isopropoxyiminoacetic acid는 Lonza사 제품을, *p*-methoxybenzyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride (ACLE) Otsuka사 제품을, silica gel(70~230 mesh)은 Sigma사 제품을 사용하였고 용매는 필요에 따라 정제하여 사용하였다. Muller-Hinton broth는 Difco co., 제품을 사용하였다. Thin layer chromatography (TLC)는 Kieselgel F_{254}(0.25 mm)를 바른 유리판을 잘라 이용하였으며 tlc spot는 자외선램프 UVGL-58을 사용하였다. 융점 측정은 Gallen-Kamp melting point apparatus를 사용하였으며, 이에 대한 보정은 하지 않았다. Column chromatography는 silica gel(230~400 mesh, 60 Å, Merck)을 사용하였다. NMR spectra는 tetramethylsilane(TMS)를 내부 표준물질로 하여 Bruker FT-80 MHz, FT-300 MHz를 사용하였다.

p-Methoxybenzyl 7β[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (2)



Scheme 1 – Synthesis of compounds (**2**).



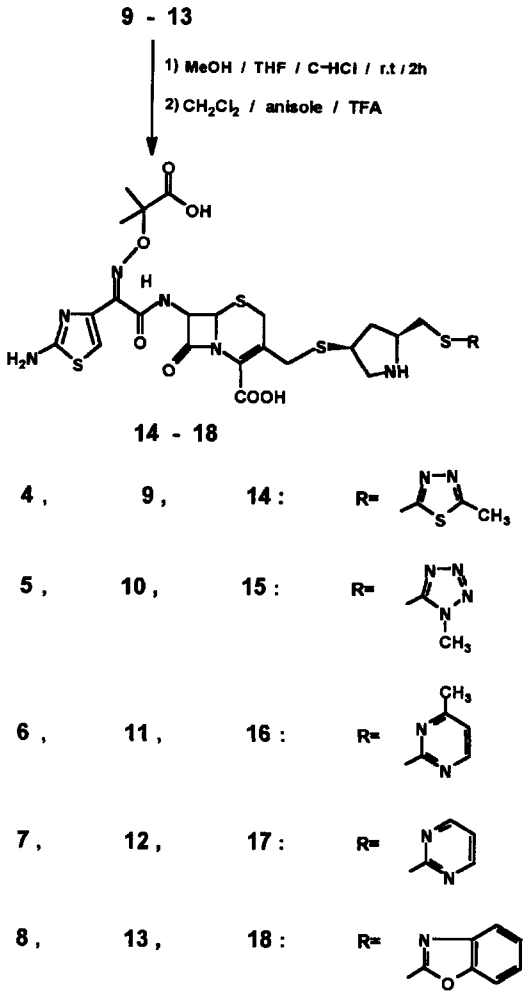
Scheme 2 – Synthesis of compounds (**9-13**).

DMF 2 m*l*와 THF 20 m*l*의 혼합용액에 $POCl_3$ 1.5 m*l*(17 mmol)을 가하여 −5~0°C에서 30분간 교반시킨 후 화합물 **1** 5 g(14 mmol)을 가하고 −15~−20°C에서 1시간 동안 교반하였다. 한편 ACLE 4.85 g(12 mmol)을 EtOAc 20 m*l*에 현탁시키고 BSA 10 m*l*(40 mmol)을 가하여 30분간 교반시킨 후 −15~−20°C에서 위의 반응액에 가하고 30분간 교반시켰다. 반응물을 EtOAc로 추출하고 5% $NaHCO_3$ 수용액과 brine으로 세척한 후 무수 $MgSO_4$로 건조한 다음 여액을 감압 농축시키고 isopropylether(IPE)에 분산 여과하여 백색결정 7.2 g(86.7%)을 얻었다. 이것을 소량의 Me_2CO에 녹인 후 column chromatography (EtOAc/hexane=1:2)로 정제하여 6.4 g(77.1%)을 얻었다.

mp : 115~118°C

Rf : 0.58(EtOAc/hexane=1:1)

^{1}H-NMR(DMSO-d_6) δ : 1.38(9H, s, $(CH_3)_3$), 1.43 (6H, s, $(CH_3)_2$), 3.52(2H, m, C_2-H), 3.75(3H, s, OCH_3), 4.52(2H, dd, J=12 Hz, CH_2Cl), 5.12~5.25(3H, m, OCH_2, C_6-H), 5.93(1H, dd, J=5 Hz, C_7-H), 6.92(2H, d, J=5 Hz, ArH), 7.36(3H, m, ArH, thiazole-H), 8.48(1H, s, HCONH), 9.56(1H, d, J=8 Hz, CONH), 12.67(1H, s, HCO)



Scheme 3 – Synthesis of compounds (**14-18**).

(2*S*, 4*S*)-4-Acetylthio-2-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-1-*tert*-butoxycarbonylpyrrolidine (4)

(2*S*, 4*S*)-4-Acetylthio-2-iodomethyl-1-*tert*-butoxy-carbonylpyrrolidine(**3**) 2.0 g(5.2 mmol) 을 Me_2CO 40 m*l* 에 녹인 다음 K_2CO_3 1.08 g(7.8 mmol) 과 5-methyl-1,3,4,-thiadiazole-2-thiol 0.76 g(5.6 mmol)을 가한 후 6시간 동안 환류 교반시켰다. 반응물을 냉각하고 감압 농축시킨 후 EtOAc로 추출한 다음 증류수, sat. $NaHCO_3$ 용액, brine 순으로 세척한 후에 유기층을 무수 $MgSO_4$로 건조시키고 감압 농축하여 황갈색 액체 2.16 g을 얻었다. 이 물질을 소량의 EtOAc에 녹이고 column chromatography(EtOAc/hexane=1:4) 로 정제하여 미황색 액체 1.96 g(96.9%) 을 얻었다.

Rf : 0.43 (EtOAc/hexane=1:2)

^{1}H-NMR($CDCl_3$) δ : 1.47(9H, s, $(CH_3)_3$), 1.93 ~ 2.04(1H, m, C_3-H), 2.34(3H, s, CH_3CO), 2.58(1H, m, C_3-H), 2.72(3H, s, thiadiazole -CH_3), 3.13 ~ 3.19 (1H, m, C_5-H), 3.49 ~ 3.91(5H, m, CH_2, C_2-H, C_4-H, C_5-H)

(2*S*, 4*S*)-4-Acetylthio-2-(1-methyltetrazol-2-yl) thiomethyl-1-*tert*-butoxycarbonylpyrrolidine (5)

화합물**5**~**8**는 화합물**4**와 같은 방법으로 합성하였다.

수득율 : 82.1%

Rf : 0.44(EtOAc/hexane=1:2)

^{1}H-NMR($CDCl_3$) δ : 1.47(9H, s, $(CH_3)_3$), 1.88~ 1.98(1H, m, C_3-H), 2.34(3H, s, CH_3CO), 2.56~ 2.59(1H, m, C_3-H), 3.14 ~ 3.20(1H, m, C_5-H), 3.61~ 4.20(4H, m, CH_2, C_4-H, C_5-H), 3.95(3H, s, N-CH_3), 4.25(1H, m, C_2-H)

(2*S*, 4*S*)-4-Acetylthio-2-(4-methylpyrimidin-2-yl) thiomethyl-1-*tert*-butoxycarbonylpyrrolidine (6)

수득율 : 90.5%

Rf : 0.54(EtOAc/hexane=1:2)

^{1}H-NMR($CDCl_3$) δ : 1.48(9H, s, $(CH_3)_3$), 1.90~ 1.98(1H, m, C_3-H), 2.32(3H, s, CH_3CO), 2.45(3H, s, pyrimidine-CH_3), 2.54~2.56(1H, m, C_3-H), 3.18(1H, dd, *J*=7.8 Hz, C_5-H), 3.75~4.05(4H, m, CH_2, C_5-H, C_4-H), 4.22~4.24(1H, m, C_2-H), 6.83(1H, d, *J*= 5.1 Hz, ArH), 8.41(1H, d, *J*=5.1Hz, ArH)

(2*S*, 4*S*)-4-Acetylthio-2-(pyrimidin-2-yl)thiomethyl-1-*tert*-butoxycarbonylpyrrolidine (7)

수득율 : (84.4%)

Rf : 0.49(EtOAc/hexane=1:2),

^{1}H-NMR($CDCl_3$) δ : 1.49(9H, s, $(CH_3)_3$), 1.90~ 1.94(1H, m, C_3-H), 2.33(3H, s, CH_3CO), 2.55(1H, m, C_3-H), 3.15~3.22(1H, m, C_5-H), 3.75~3.89(3H, m, CH_2, C_5-H), 4.04(1H, m, C_4-H), 4.24(1H, m, C_2-H), 6.70(1H, m, pyrimidine-H), 8.51(2H, dd, J=3 Hz, pyrimidine-H)

(2*S*, 4*S*)-4-Acetylthio-2-(benzoxazol-2-yl) thio-

methyl-1-*tert*-butoxycarbonylpyrrolidine (8)

수득율 : 88.7%

mp : 95~96°C

Rf : 0.58(EtOAc/ hexane=1:2), ^{1}H-NMR($CDCl_3$) δ : 1.47(9H, s, $(CH_3)_3$), 1.91~1.98(1H, m, C_3-H), 2.32 (3H, s, CH_3CO), 2.59(1H, m, C_3-H), 3.16~3.22 (1H, m, C_5-H), 3.85~4.20(4H, m, CH_2, C_4-H, C_5-H), 4.24~4.33(1H, m, C_2-H), 7.20~7.29(2H, m, ArH), 7.41~7.45(1H, m, ArH), 7.58~7.56(1H, m, ArH)

***p*-Methoxybenzyl 7β-[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino] acetamido-3-[(3S, 5S)-5-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-1-*tert*-butoxycarbonyl-pyrrolidin-3ylthio]methyl-3-cephem-4-carboxylate (9)**

화합물 **4** 1.8 g(4.62 mmol)을 MeOH 20 ml에 녹이고 ice-bath 상에서 1N-NaOH 3 ml를 가한 후 40분간 교반시켰다. 반응물을 EtOAc로 추출하고 1N-HCl, sat. $NaHCO_3$, brine순으로 세척하였다. 유기층을 취하여 무수 $MgSO_4$로 건조시킨 후 감압 농축시켜 미황색 액체 1.5 g(93.3%)을 얻었다. 얻어진 화합물을 정제하지 않고 바로 다음 반응에 사용하였다. 위의 화합물 1.0 g(2.88 mmol)을 취하여 CH_3ONa 0.15 g(2.88 mmol)을 MeOH 20 ml에 녹힌 용액에 -20℃에서 가하여 1시간 교반하였다. 한편 화합물 **2** 2.0 g (2.88 mmol), Me_2CO 30 ml, NaI 0.04 g(2.88 mmol), K_2CO_3을 40~45°C에서 6시간 교반하여 여과하고 여액을 위의 sodium thiolate용액에 -20°C에서 가하여 빙욕상에서 2시간 교반하였다. 반응물을 감압 농축시킨 후 EtOAc로 추출하고 증류수, sat. $NaHCO_3$ 용액, brine순으로 세척한 후 유기층을 취하여 무수 $MgSO_4$로 건조시키고 감압 농축시킨 후 이 물질을 소량의 Me_2CO에 녹여 column chromatography(EtOAc/hexane=1:2)로 정제하여 미황색 고체 1.1 g(39.1%)을 얻었다.

mp : 92~94°C

Rf : 0.54(EtOAc/hexane=1:1)

^{1}H-NMR($CDCl_3$) δ : 1.42(9H, s, $(CH_3)_3$), 1.47(9H, s, $(CH_3)_3$), 1.57(6H, s, $(CH_3)_2$), 1.95(1H, m, pyrrolidine C_3-H), 2.49(1H, m, pyrrolidine C_3-H), 2.72 (3H, s, thiadiazole-CH_3), 3.15~3.68(3H, m, C_2-H, pyrrolidine C_5-H), 3.81(2H, s, pyrrolidine C_2-CH_2), 3.82(3H, s, OCH_3), 3.91~4.16(4H, m, pyrrolidine C_2-H, pyrrolidine C_2-H, C_3-CH_2), 5.06~5.35(3H, m, C6-H, OCH_2), 5.92(1H, dd, J=5 Hz, C_7-H), 6.91(2H, d, J=8 Hz, ArH), 7.25~7.46(3H, m, thiazole-H, ArH), 8.54(1H, s, HCO)

***p*-Methoxybenzyl 7-[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino]acetamido-3-[(3*S*,5*S*)-5-(1-methyltetrazol-2-yl)thiomethyl-1-*tert*-butoxycarbonylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylate (10)**

화합물 **10~13**는 화합물 **9**와 같은 방법으로 합성하였다.

수득율 : 63.4%

mp : 89~91°C

Rf : 0.55(EtOAc/ hexane=1:1)

^{1}H-NMR($CDCl_3$) δ : 1.42(9H, s, $(CH_3)_3$), 1.44(9H, s, $(CH_3)_3$), 1.59(6H, s, $(CH_3)_2$), 1.85(1H, m, pyrrolidine C_3-H), 2.45(1H, m, pyrrolidine C_3-H), 3.14~3.20(1H, m, pyrrolidine C_5-H), 3.35~3.64(2H, m, C_2-H), 3.81(2H, s, pyrrolidine C_2-CH_2), 3.83(3H, s, OCH_3), 3.92(3H, s, N-CH_3), 4.04~4.15(4H, m, pyrrolidine C_2-H, pyrrolidine C_5-H, C_3-CH_2), 5.07~5.36(3H, m, C_6-H, OCH_2), 5.95(1H, dd, J=5 Hz, C7-H), 6.88~6.92(2H, m, ArH), 7.27~7.40(3H, m, ArH, thiazole-H), 8.54(1H, s, HCO)

***p*-Methoxybenzyl 7β-[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino] acetamido-3-[(3*S*,5*S*)-5-(4-methylpyrimidin-2-yl) thiomethyl-1-*tert*-butoxycarbonyl-pyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylate (11)**

수득율 : 53.3%

mp : 87~89°C

Rf : 0.65(EtOAc/hexane=1:1)

^{1}H-NMR($CDCl_3$) δ : 1.42(9H, s, $(CH_3)_3$), 1.46(9H, s, $(CH_3)_3$), 1.58(6H, s, $(CH_3)_2$), 2.04(1H, m, pyrrolidine C_3-H), 2.45(3H, s, pyrimidine -CH_3), 2.54(1H, m, pyrrolidine C_3-H), 3.15~3.70(3H, m, pyrrolidine C_5-H, C_2-H), 3.80(2H, s, pyrrolidine C_2-CH_2),

3.82(3H, s, OCH_3), 3.91~4.18(4H, m, pyrrolidine C_2-H, pyrrolidine C_4-H, C_3-CH_2), 5.05~5.35(3H, m, C_6-H, OCH_2), 5.92(1H, dd, J=5 Hz, C7-H), 6.83~6.92(3H, m, ArH), 7.27~7.40(3H, m, ArH, thiazole-H), 8.34(1H, m, ArH), 8.54(1H, s, HCO)

***p*-Methoxybenzyl 7β-[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino] acetamido-3-[(3*S*, 5*S*)-5-(pyrimidin-2-yl)thiomethyl-1-*tert*-butoxycarbonylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxy-late (12)**

수득율 : 38.9%

mp : 90~93°C

Rf : 0.57(EtOAc/hexane=1:1)

^{1}H-NMR($CDCl_3$) δ : 1.43(9H, s, $(CH_3)_3$), 1.48(9H, s, $(CH_3)_3$), 1.56(6H, s, $(CH_3)_2$), 1.88~1.92(1H, m, pyrrolidine C_3-H), 2.47(1H, m, pyrrolidine C_3-H), 3.15~3.75(3H, m, pyrrolidine C_5-H, C_2-H), 3.79 (2H, s, pyrrolidine C_2-CH_2), 3.81(3H, s, OCH_3), 3.91~4.15(4H, m, pyrrolidine C_2-H, pyrrolidine C_5 -H, C_3-CH_2), 5.04~5.25(3H, m, C_6-H, OCH_2), 5.81 (1H, dd, J=5 Hz, C7-H), 6.82(1H, d, J=5 Hz, ArH), 6.79~6.95(3H, m, pyrimidine-H, Ar-H), 7.31~ 7.40(3H, m, ArH, thiazole-H), 8.48~8.55(3H, m, HCO, pyrimidine-H)

***p*-Methoxybenzyl 7β-[(Z)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylisopropoxyimino]acetamido-3-[(3*S*, 5*S*)-5-(benzoxazol-2-yl) thiomethyl-1-*tert*-butoxycarbonylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylate (13)**

수득율 : 59.4%

mp : 87~88°C

Rf : 0.72(EtOAc/hexane=1:1)

^{1}H-NMR($CDCl_3$) δ : 1.42(9H, s, $(CH_3)_3$), 1.46(9H, s, $(CH_3)_3$), 1.56(6H, s, $(CH_3)_2$), 1.92~1.98(1H, m, C_3-H), 2.45(1H, m, pyrrolidine C_3-H), 3.16(3H, m, C_2-H, pyrrolidine C_5-H), 3.80(2H, s, pyrrolidine C_2-CH_2), 3.81(3H, s, OCH_3), 3.89~4.20(4H, m, pyrrolidine C_2-H, pyrrolidine C_5-H, C_3-CH_2), 5.05~ 5.35(3H, m, C_6-H, OCH_2), 5.93 (1H, dd, J=5 Hz, C_7-H), 6.89(2H, d, J=8 Hz, ArH), 7.20~7.59(7H, m, ArH, benzoxazole-H, thiazole-H), 8.53(1H, s, HCO)

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (14)

화합물 **9** 1.1 g(1.09 mmol)을 MeOH 9 m*l*와 THF 1.75 m*l*에 녹이고 c-HCl 0.37 m*l*을 가하여 실온에서 2시간 동안 교반시킨 후 5% $NaHCO_3$ 용액으로 중화시키고 감압 농축하였다. 이 잔사에 EtOAc 50 m*l*와 증류수 50 m*l* 를 가하고 10% HCl 로 pH 2.0 로 맞춘 후 유기층을 취하였다. 유기층을 무수$MgSO_4$로 건조시키고 감압 농축시킨 후 이 물질을 소량의 Me_2CO에 녹이고 column chromatography(EtOAc/hexane =1:1.5)로 정제하여 미황색 고체 0.6 g(56.1%)을 얻었다. 위의 화합물을 ice-bath상에서 CH_2Cl_2 7 m*l*에 녹히고 여기에 anisole 2.0 m*l*(18 mmol) 과 TFA 7.1 m*l*(92 mmol)를 서서히 가해준다. 실온에서 2시간 동안 교반한 후 감압 농축시키고 IPE로 결정화 하였다. 이것을 중조용액에 녹힌후 EtOAc, CH_2Cl_2으로 세척한 후 물층을 냉동 건조하였다. 이 결정을 소량의 물에 녹인 후 column chromatography(H_2O/AcCN=1:5)로 정제하여 미백색의 고체 0.12 g(25%) 을 얻었다.

mp : 203~204°C

Rf : 0.42(CH_3CN/H_2O=3:1)

^{1}H-NMR(DMSO-d_6) δ : 1.49(6H, s, $(CH_3)_2$), 1.83 (1H, m, pyrrolidine C_3-H), 2.48(1H, m, pyrrolidine C_3-H), 2.81~4.19(13H, m, thiadiazole-CH_3, C_2-H, C_3-CH_2, pyrrolidine C_2-H, pyrrolidine C_4-H, pyrrolidine, C_5-H, pyrrolidine C_2-CH_2), 5.20(1H, m, C_6-H), 5.88 (1H, dd, C_7-H), 6.89(1H, s, thiazole-H), 9.56(1H, d, J=8 Hz, CONH)

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(1-methyltetrazol-2-yl)thiomethylpyrrolidin-3-ylthio] methyl-3-cephem-4-carboxylic acid (15)

화합물 **15~18**는 화합물 **14**와 같은 방법으로 합성하였다.

수득율 : 26.6%

mp : 209~211°C

Rf : 0.42(CH_3CN/H_2O=3:1)

^{1}H-NMR(DMSO-d_6) δ : 1.48(6H, s, $(CH_3)_2$), 1.80 (1H, m, pyrrolidine C_3-H), 2.43(1H, m, pyrrolidine C_3-H), 2.85~3.85(7H, m, pyrrolidine C_4-H, pyrrolidine C_5-H, pyrrolidine-CH_2, C_2-H), 3.89(3H, m, tetrazole-CH_3), 4.04~4.20(3H, pyrrolidine C_2-H, C_3 -CH_2), 5.22(1H, m, C_6-H), 5.91(1H, m, C_7-H), 6.88 (1H, s, thiazole-H), 9.55(1H, d, J=8 Hz, CONH)

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(4-methylpyrimidin-2-yl)thiomethylpyrrolidin-3-ylthio] methyl-3-cephem-4-carboxylic acid (16)

수득율 : 47.4%

mp : 227 ~ 228°C

Rf : 0.47(CH_3CN/H_2O=3:1)

^{1}H-NMR(DMSO-d_6) δ : 1.44(6H, s, $(CH_3)_2$), 1.82 (1H, m, pyrrolidine C_3-H), 2.47~2.52(4H, m, pyrimidine-CH_3, pyrrolidine C_5-H), 2.98~3.85(7H, m, C_2-H, pyrrolidine C_4-H, pyrrolidine C_5-H, pyrrolidine-CH_2), 5.21(1H, m, C_6-H), 5.76~5.95(1H, m, C_7-H), 6.81~6.91(2H, m, pyrimidine-H, thiazole-H), 8.43(1H, m, pyrimidine-H), 9.55(1H, d, J=8 Hz, CONH)

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(pyrimidin-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (17)

수득율 : 48.3%)

mp : 212~215°C

Rf : 0.45(CH_3CN/H_2O=3:1)

^{1}H-NMR(DMSO-d_6) δ : 1.35(6H, s, $(CH_3)_2$), 1.80 (1H, m, pyrrolidine C_3-H), 2.47(1H, m, pyrrolidine C_3-H), 2.91~3.02(1H, m, pyrrolidine C_5-H), 3.25~3.72(6H, m, C_2-H, pyrrolidine C_4-H, pyrrolidine C_5-H, pyrrolidine-CH_2), 4.05~4.26(3H, m, pyrrolidine C_2-H, C_3-CH_2), 5.25(1H, m, C6-H), 5.84(1H, dd, J=5 Hz, C_7-H), 6.79~6.95(2H, m, pyrimidine-H, thiazole-H), 8.51(2H, m, pyrimidine-H), 9.51 (1H, d, J=8 Hz, CONH)

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(benzoxazol-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (18)

수득율 : 32.8%

mp : 205~208°C

Rf : 0.48(CH_3CN/H_2O=3:1)

^{1}H-NMR(DMSO-d_6) δ : 1.55(6H, s, $(CH_3)_2$), 1.90 (1H, m, pyrrolidine C_3-H), 2.50(1H, m, pyrrolidine C_3-H), 2.95~3.70(7H, m, pyrrolidine C_4-H, pyrrolidine C_5-H, pyrrolidine-CH_2, C_2-H), 4.05~4.28(3H, m, pyrrolidine C_2-H, C_3-CH_2), 5.25(1H, m, C_6-H), 5.90(1H, m, C_7-H), 6.88(1H, s, thiazole-H), 7.20~7.59(4H, m, Ar-H), 9.55(1H, d, J=8Hz, CONH)

항균력실험

시험균주

Bacillus subtilis ATCC6633, *Staphylococcus aureus* KCTC9341, *Micrococcus luteus* ACTCC9341, *Escherichia coli* AB1157, *Escherichia coli* AB0119, *Salmonella typhimurium* TV119, *Salmonella typhimurium* SL1102, *Pseudomonas aeruginosa* KCTC-1637, *Alcaligenes faecalis* KCTC1004, *Candida al-*

Table I – MICs(μg/ml) of synthetic compounds and commercial antibiotics against representative microorganisms

Strain \ Compounds	14	15	16	17	18	S
Bacillus subtilis ATCC6633	20	5	5	5	20	0.08
Staphylococcus aureus KCTC9341	20	20	20	20	20	1.25
Micrococcus luteus ACTCC9341	20	10	20	2.5	40	0.32
Escherichia coli AB1157	5	5	20	5	20	0.63
Escherichia coli AB0119	40	2.5	5	20	40	0.08
Salmonella typhimurium TV119	20	2.5	5	40	20	20
Salmonella typhimurium SL1102	20	20	40	20	20	2.5
Pseudomonas aeruginosa KCTC1637	40	5	10	40	40	0.63
Alcaligenes faecalis KCTC1004	20	2.5	2.5	20	40	10
Candida albicans ATCC10231	>40	>40	>40	>40	>40	>40

S : cefotaxime

bicans ATCC10231

배 지

시험 균주의 전배양 및 검정 plate의 제조 목적으로 Muller Hinton broth(DIF Co.) 을 사용하였다.

항균 활성 측정법

시험균의 전배양 – *Bacillus subtilis* ATCC6633 및 9개의 균주를 nutrient agar 배지에 37°C에서 12시간 배양한 후, nutrient agar 배지에서 얻은 single colony를 LB배지 20 m*l*에 접종한 후, 37°C에서 12시간 배양하였다.

검정 plate의 제조 – 화합물 **14, 15, 16, 17, 18**를 소량의 증류수에 녹인 후 각각의 시료 1 m*l*를 2단계 희석법으로 14차례 희석하여 영양 한천 배지 14 m*l*와 섞었을 때, 최종배지의 화합물 **14, 15, 16, 17, 18** 및 대조물질 (cefotaxime) 의 농도가 40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08, 0.04, 0.02, 0.01, 0.005 μg/m*l* 이 되도록 제조하였다.

항균력 판정 – 각각의 시험균주들을 검정 plate에 접종한 것을 37±2°C에서 18시간 배양 후 2배씩 단계적으로 희석하여 접종한 plate를 일렬로 나열하여 육안으로 관찰하여 성장이 억제된 항균제의 최소 발육저지 농도 (Minimum Inhibitory Concentration, MIC)를 조사하여 **Table I** 과 같은 결과를 얻었다.

결 론

Cephem ring 의 7 위치에는 (Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetyl group을, 3위치에는 heterocyclic-pyrrolidine moiety를 합성하여 도입시킨 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3*S*, 5*S*)-5-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid(**14**), 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(1-methyltetrazol-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid(**15**), 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino] acetamido-3-[(3*S*, 5*S*)-5-(4-methylpyrimidin-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (**16**), 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino]acetamido-3-[(3S, 5S)-5-(pyrimidin-2-yl)thiomethylpyrrolidin-3-ylthio]methyl-3-cephem-4-carboxylic acid (**17**) 및 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino] acetamido-3-[(3*S*, 5*S*)-5-(benzoxazol-2-yl)thiomethylpyrrolidin-3-ylthio] methyl-3-cephem-4-carboxylic acid (**18**) 을 각각 합성하였다. 합성한 화합물들을 Gram(+), Gram(–) 균에 대하여 항균력을 실험한 결과 대체적으로 대조물질인 cefotaxime보다 저하되었다. 그러나 pyrrolidine핵에 1-methyltetrzole을 도입시킨 화합물 **15** 은 *Salmonella typhimurium* TV119 및 *Alcaligenes faecalis* KCTC1004 균에 대해서는 cefotaxime보다 각각 8배 및 4배의 항균작용을 나타냈으며, *Salmonella typhimurium* TV119에 대한 항균력은 cefotaxime 보다 좋았으나 동일종인 *Salmonella typhimurium* SL1102에 대한 항균력이 오히려 저하되었는데 이는 이들 두 균주의 세포벽구조상의 차이점에 기인한 것으로 사려된다. 4-methyl pyrimidine을 도입시킨 화합물 **16** 는 *Alcaligenes faecalis* KCTC1004 균에 대하여 cefotaxime 보다 4 배의 항균력을 보여 주었다.

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