THE SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL AMINOHETEROCYCLIC METHOXIME MONOBACTAM DERIVATIVES

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Two novel monobactams, 3-β-[2-(3-aminooxazol-4-yl)-2-*Z*-(methoximinoacetamido)]-4-α-methyl-2-oxoazetidine-1-sulfonic acid (**4**) and 3-β-[2-(5-aminooxadiazol-3-yl)-2-*Z*-(methoximinoacetamido)]-4-α-methyl-2-oxoazetidine-1-sulfonic acid (**5**) were synthesized and evaluated microbiologically. Although less active than the corresponding aminothiazole **6** and aztreonam against Gram-negative bacteria **4** was found to be more active than either **6** or aztreonam against Streptococci. The aminooxadiazole **5** was the least active compound tested in this series.

In the past few years, since SQ26445 was isolated from *Pseudomonas acidophilia*[1] and the subsequent synthesis of 3-AMA and the corresponding 4-methyl analogs[2], numerous reports concerning the structure-activity relationship of this unique class of β-lactam antibiotics have appeared[3~5].

Recently several papers concerning the synthesis and microbiological evaluation of novel aminoheterocyclic methoxime cephalosporins have appeared[6~9]. Since the microbiological activity of monobactam antibiotics has been far from optimized, we have undertaken the synthesis of aminooxazolyl- and aminooxadiazolylmethoxime substituted monobactams in hopes of achieving more of a balance between the activities against Gram-positive and Gram-negative bacteria. The results of these investigations are reported herein.

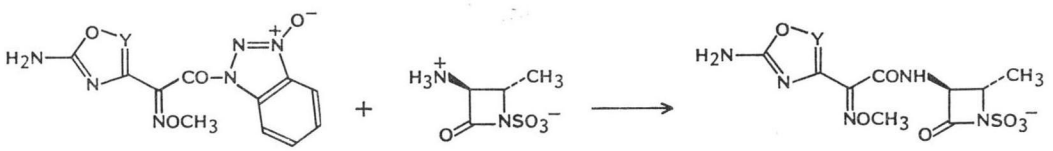
Chemistry

Utilizing HBT-active esters (**1** and **2**) which have been previously described[8,9], the 3-aminomonobactamic acid **3** was acylated under modified Schotten-Baumann conditions (Scheme 1).

Biological Results

Antimicrobial activities of **4** and **5** as well as **6**[5] and aztreonam[4] (included as reference compounds) were determined in an agar dilution assay (Table 1). Activities were determined against a wide variety of Gram-positive and Gram-negative aerobic bacteria (Table 1). All compounds tested were inactive against both penicillin-sensitive and -resistant *Staphylococcus aureus* as well as *Staphylococcus epidermidis*. The activities of **4** and **6** were substantially better against *Streptococcus pyogenes* and *Streptococcus pneumoniae* than the other compounds tested. While **5** was more active than aztreonam, it was 2 to 8-fold less active than **4** and **6** against those 2 strains. All of the compounds tested

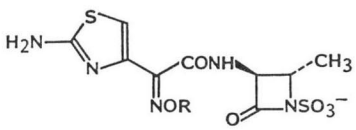
Scheme 1.



1 Y = CH
2 Y = N

3

4 Y = CH
5 Y = N



Aztreonam R = $C(CH_3)_2COOH$
6 R = CH_3

Table 1. *In vitro* antibacterial activity.

Species	Strain	Agar dilution MIC (μg/ml)			
		4	**5**	**6**	Aztreonam
Staphylococcus aureus	X1.1	>128	>128	>128	>128
S. aureus	V41[a]	>128	>128	>128	>128
S. aureus	X400[a,b]	>128	>128	>128	>128
S. aureus	S13E[a,b]	>128	>128	>128	>128
S. epidermidis	Epi 1[a,b]	>128	>128	>128	>128
S. epidermidis	222[a]	128	>128	64	>128
Streptococcus pyogenes	C203	2	8	4	16
S. pneumoniae	PARK I	1	8	2	64
Enterococcus faecalis	X66	>128	>128	>128	>128
E. faecalis	9960	>128	>128	>128	>128
Haemophilus influenzae	C.L.	8	16	0.5	0.06
H. influenzae	76[c]	2	8	0.25	0.06
Escherichia coli	N10	1	32	0.06	0.125
E. coli	EC14	0.5	8	0.03	0.03
E. coli	TEM[e]	4	32	0.125	0.06
Shigella sonnei	N9	1	16	0.06	0.06
Klebsiella pneumoniae	X26	1	16	0.03	0.06
K. pneumoniae	KAE[d]	>128	>128	>128	32
K. pneumoniae	X68	1	16	0.06	0.03
Enterobacter aerogenes	C32	1	16	0.06	0.06
E. aerogenes	EB17	1	32	0.06	0.06
E. cloacae	EB5	2	64	0.125	0.06
E. cloacae	265A[e]	64	128	32	32
Salmonella typhi	X514	1	16	0.06	0.06
S. typhi	1335	2	16	0.125	0.125
Pseudomonas aeruginosa	X528	>128	128	128	4
P. aeruginosa	X239	>128	128	64	4
P. aeruginosa	PS18[f]	>128	>128	>128	64
P. aeruginosa	PS72	>128	>128	128	8
Serratia marcescens	X99	4	16	0.25	0.125
S. marcescens	SE3	4	64	0.25	0.25
Morganella morganii	PR15	8	64	1	0.03
Providencia stuartii	PR33	4	8	0.25	0.03
P. rettgeri	C24	0.25	8	0.03	0.015
Citrobacter freundii	CF17	1	16	0.06	1
Acinetobacter calcoaceticus	AC12	8	32	4	32

[a] β-Lactamase producer.
[b] Methicillin-resistant.
[c] TEM (Type 3) β-lactamase producer.
[d] Type IVc β-lactamase producer.
[e] Constitutive Type 1 high level β-lactamase producer.
[f] Type Id β-lactamase producer.

were inactive against Enterococci.

Against cephalothin-sensitive Enterobacteriaceae, aztreonam was the most active compound tested. Aminothiazole derivative **6** was usually found to be 1~2 dilutions less active against these bacteria, while aminooxazole derivative **4** was 3~4 dilutions less active than **6**. The aminooxadiazole derivative **5** was significantly less active than any of the compounds tested (MICs ranging from 8 to 64 μg/ml). This has been the trend observed in the other series examined[8,9] (aminothiazole>aminooxazole>aminooxadiazole).

This trend continued when the compounds were tested against the more resistant bacteria. Aztreonam was the only compound which

showed activity against *Pseudomonas aeruginosa*, while the activities of aztreonam and **6** against resistant Enterobacteriaceae were similar. While **4** possessed useful activity it was generally 3~4 dilutions less active than **4** against these bacteria. The activity of **5** was significantly less.

Experimental

NMR spectra were recorded on a Varian Associates EM-390 (90 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. All melting points are uncorrected. Agar dilution MICs were determined by the method described in KIRST *et al.*[10].

3-[2-β-(2-Aminooxazol-4-yl)-2-*Z*-methoximinoacetamido]-4-α-methyl-2-oxoazetidine-1-sulfonic Acid, Sodium Salt (**4**)

A 50% aqueous acetone suspension of **3**[5] (0.360 g, 2 mmol) was neutralized to pH 6.8 by the dropwise addition of 1 N NaOH. The resulting solution was stirred and **1**[9] (0.700 g, 2.08 mmol) was added. Stirring was continued while the pH of the solution was maintained between 6.8 and 7.0 by the addition of 0.1 N NaOH. The active ester **1** was slowly consumed and solution was complete after 1 hour, whereupon stirring was continued an additional 2 hours.

The acetone was removed *in vacuo* and the resulting aqueous solution was washed twice with EtOAc. The aqueous layer was concentrated and crystallization of 1-hydroxybenzotriazole began. After allowing to stand overnight, the solution was filtered and the filtrate was evaporated to dryness. The residue was re-dissolved in warm EtOH and chilled to effect crystallization. The crystals were filtered to yield **4** as a white solid (0.1 g, 13.6%), mp 175~180°C (dec). *Anal* Calcd for $C_{10}H_{12}N_5O_7SNa$: C 32.52, H 3.28, N 18.96. Found: C 31.17, H 3.77, N 17.34. NMR (DMSO-d_6) δ 1.46 (3H, d, J=6 Hz, 4-CH_3), 3.69 (1H, dd, J=3 and 6 Hz, 4-CH), 3.88 (3H, s, OCH_3), 4.36 (1H, dd, J=3 and 7.5 Hz, 3-CH), 4.50 (2H, br s, NH_2), 7.79 (1H, s, oxazole-H) and 9.38 (1H, d, J=7.5 Hz, CONH).

3-[2-β-(3-Amino[1,2,4]oxadiazol-4-yl)-2-*Z*-methoximinoacetamido]-4-α-methyl-2-oxoazetidine-1-sulfonic Acid, Sodium Salt (**5**)

A 50% aqueous suspension of **3**[5] (0.54 g, 3 mmol) was treated as described above with **2**[8] (1.01 g, 3 mmol). After work-up, the aqueous layer was evaporated and the residue was dissolved in warm EtOH and filtered. From the filtrate, **5** precipitated as a crude amorphous solid (0.415 g). Crystallization from EtOH yielded **5** as a white solid, 0.153 g (13.7%), mp 195°C (dec). *Anal* Calcd for $C_8H_{11}N_6O_7SNa$ (EtOH): C 31.71, H 4.08, N 20.18. Found: C 31.47, H 3.76, N. 20.35. NMR (DMSO-d_6) δ 1.35 (3H, d, J=9 Hz, 4-CH_3), 3.60 (1H, m, 4-CH), 3.75 (3H, s, OCH_3), 4.39 (1H, dd, J=3 and 9 Hz, 3-CH), 7.98 (2H, s, NH_2) and 9.40 (1H, d, J=9 Hz, CONH). In addition the NMR indicated the presence of 1 mol of EtOH.

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