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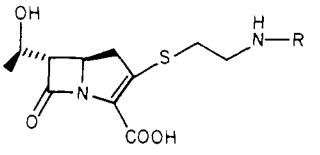
Communications to the Editor

N-Acetimidoyl- and *N*-Formimidoylthienamycin Derivatives: Antipseudomonal β-Lactam Antibiotics

Sir:

Thienamycin (1) is a recently discovered β-lactam antibiotic of unique structure and exceptional breadth of antibacterial activity. Of particular interest is its exceptional potency against *Pseudomonas spp.* and its β-lactamase stability.[1] The novel carbapenem nucleus and aminoethylthio side chain of thienamycin, however, contribute to its chemical instability. For example, in comparing the stability of thienamycin to benzylpenicillin, comparable stability exists only between pH 2 and 5. Near pH 7, the most stable pH for thienamycin, benzylpenicillin is more stable. Also, the stability of thienamycin is inversely related to its concentration. This latter characteristic makes thienamycin less attractive for use as a parenterally administered drug.



1, R = H
2, R = CH=NH
3, R = C(CH$_3$)=NH

It has been postulated that the concentration-dependent instability of thienamycin is due to the intermolecular aminolysis of the azetidinone by the cysteamine side chain.[2] Derivatization of the amino group of thienamycin to a less nucleophilic species seems an attractive route to more stable thienamycin analogues. Since the naturally occurring *N*-acetyl derivative[3] has greatly diminished antipseudomonal activity, retention of antipseudomonal activity appeared to require a basic functionality. It was therefore considered likely that conversion of the amine to a stronger base would, by virtue of its existence to a greater extent in a protonated form, result in a compound with increased stability in concentrated solution as well as high antipseudomonal activity. We also hoped to obtain a crystalline derivative which would facilitate purification. We now report that the *N*-acetimidoyl (3) and particularly the *N*-formimidoyl (2) derivatives fulfilled these expectations.

The amidines are readily prepared by the reaction of thienamycin with an imidate ester in aqueous solution at pH 8.2. The reaction mixtures were adjusted to pH 7 and chromatographed at 4 °C on a column of Dowex 50 × 4 (Na^+ cycle, 200–400 mesh) resin eluted with deionized water. The amidines are retarded relative to thienamycin and are obtained as amorphous powders on lyophilization of the appropriate fractions. Thus, the reaction of 1 with methyl formimidate gave 2 (45% yield): UV (H_2O) λ_{max} 298 nm (ε 8040, 99% NH_2OH ext); IR (Nujol mull) 1767 cm^{-1} (β-lactam); NMR (D_2O, 100 MHz) δ 1.30 (d, J = 6 Hz, CH_3), 2.7–3.6 (m, 4, CH_2, SCH_2), 3.40 (dd, J = 3 and 6 Hz, H-6), 3.5–3.7 (m, 2, NCH_2), 4.1–4.4 (m, 2, CHOH, H-5), 7.81 (s, 1, CHNH). Compound 2 was crystallized from water–ethanol, giving a monohydrate: UV (H_2O) λ_{max} 299 nm (ε 9670, 98% NH_2OH ext); $[\alpha]^{25}_D$ +86.8° (c 0.05, 0.1 M PO_4, pH 7). Anal. ($C_{12}H_{17}N_3SO_4$) C, H, N, S.

Table I. Solution and Solid-State Stability[a]

no.	t_{10}, h,[b] at 23 °C: concn, mg/mL	deionized H_2O	1 M PO_4, pH 7	solid,[c] month (%)
1	60	0.5	0.5	1 (80)
2	60	2.5	0.5	6 (80)
2 (cryst)	10[d]	17		6 (100)
3	60	5.0	0.5	5 (100)

[a] Based on NH_2OH extinguished UV absorbance (ref 2). [b] t_{10} = time at which 90% of initial UV absorbance remains. [c] Kept over Drierite at room temperature; amount remaining at given time. [d] Buffered with 0.1 equiv of $NaHCO_3$.

The reaction of 1 with ethyl acetimidate yielded 3 (57%): UV (H_2O) λ_{max} 298 nm (ε 8,350, 99% NH_2OH ext); IR (Nujol mull) 1774 cm^{-1} (β-lactam); NMR similar to 2 except δ 2.24 (s, 3, CH_3C=NH) replacing δ 7.81; $[\alpha]^{25}_D$ +74.6° (c 0.05, 0.1 M PO_4, pH 7). The purity of the lyophilized amidines as measured by hydroxylamine extinguished UV absorbance compares favorably with that of thienamycin (ε 7900, 95% NH_2OH ext). Additionally high-performance LC[4] analyses show 2 to be 97% and 3 >99% single components. All lyophilized samples of 2 contain 2–3% of 1, indicating that some hydrolysis of the formamidine occurs during isolation. Crystalline 2 contained <1% thienamycin.

Preliminary studies (Table I) showed the amorphous amidines to be five to ten times more stable at high concentration than a thienamycin sample of equivalent purity and represents a rough approximation of the effect of the chemical modification. An additional factor was gained from the crystallization of the *N*-formimidoyl derivative. This showed less than 1%/h decomposition at 10 mg/mL, the projected concentration for parenteral administration. As was expected, there was no change in the intrinsic sensitivity of the lactam to attack by phosphate buffer. Both amidines also show an improved shelf life.

Furthermore, these amidines retain the antibacterial spectrum of thienamycin and exhibit enhanced activity against *Pseudomonas aeruginosa* (Table II). Extensive

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Table II. Inhibitory Zone Diameters (in mm)[a] vs. Penicillin-Sensitive and -Resistant Bacterial Strains

no.	S. aureus		E. coli		Enterobacter clocae		Pseudomonas aeruginosa	
(25 μg/disk)	MB2985	MB2314	MB2482	MB2964	MB2647	MB2646	MB2835	MB3350
1	40	41	28.5	29	25.5	27	26	26
2	40	40.5	28.5	29.5	27	27.5	29	29
3	38.5	40	28	29.5	27	27	28	28

[a] In this assay a 2-mm difference in zone size corresponds approximately to a twofold difference in activity.

evaluations of in vitro antibacterial activity have shown a two- to fourfold increased potency against these species.[5] Because of its improved stability and enhanced antipseudomonal activity, *N*-formimidoylthienamycin (**2**) has been selected for clinical studies.

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References and Notes

(1) H. Kropp, J. S. Kahan, F. M. Kahan, J. Sundelof, G. Darland, and J. Birnbaum, "Thienamycin, A New β-lactam Antibiotic. II. In Vitro and In Vivo Evaluation", presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., Oct. 1976.
(2) J. S. Kahan, F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochales, S. Hernandez, H. B. Woodruff and J. Birnbaum, *J. Antibiot.*, **32**, 1 (1979).
(3) J. S. Kahan, F. M. Kahan, R. T. Goegelman, E. O. Stapley, and S. Hernandez, German Offen. 2652681, 1977.
(4) Reverse-phase high-performance LC analyses were performed using a Waters Associates high-pressure liquid chromatograph equipped with a 2.5 mm i.d. × 61 cm μBondapak C_{18}/Corasil column. **1**, **2**, and **3** had retention times of 5, 6, and 7 min, respectively, at a flow rate of 1.0 mL/min using 10% THF–H_2O as solvent.
(5) H. Kropp, J. B. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum, "MK0787 (*N*-Formimidoyl-Thienamycin): Evaluation of In Vitro and In Vivo Activity", presented at the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, Mass., Oct., 1979.

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Articles

Oxazepam Esters. 1. Correlation between Hydrolysis Rates and Brain Appearance of Oxazepam

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Esters of the centrally acting oxazepam were investigated to find quantitative correlations between the pharmacokinetics of the parent drug and in vitro biotransformation rates and physicochemical properties of its prodrugs. The ^{14}C-labeled aliphatic and ω-phenyl-substituted esters were administered intravenously to mice. Brain levels of the esters and oxazepam were determined and the latter was fitted to a simplified exponential equation. In vitro hydrolysis rate of the esters catalyzed by the hepatic microsomal fraction was measured with a pH stat. Pharmacokinetic constants characterizing the rising part of oxazepam brain levels correlate well with the chromatographic R_M values and with in vitro maximal hydrolysis rates of the esters. The hydrolysis is capacity limited in the liver. In a closely related set of aliphatic esters, oxazepam brain penetration also correlates with the steric constant (E_S) of its esters.

Prodrugs are known to modulate the pharmacokinetic properties of the parent compound.[1,2] This approach proved to be useful in drug design.[3,4]

Only few attempts have been made at defining overall pharmacokinetic–structure relationships.[4-8] There have been, however, numerous reports wherein one pharmacokinetic property—often examined in model systems in vitro—has been correlated with physicochemical parameters.[9-21]

Quantitative aspects of the correlation between chemical structure and pharmacological activity (QSAR) have been thoroughly studied. Notari[5] proposed the inclusion of the pharmacokinetic aspect to structure–activity relationships (SPAR). This is especially justified for prodrugs because activity is directly dependent on biotransformation and pharmacokinetics, the prodrug having no activity of its own.

This study on oxazepam esters as prodrugs illustrates the quantitative correlation between chemical structure and brain penetration. Oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-3*H*-1,4-benzodiazepin-2-one) is a potent tranquillizer and anticonvulsant. Although substitution with bulky moieties in position 3 was stated to diminish the effect of the 1,4-benzodiazepines on the CNS,[22] oxazepam esters as bioreversible derivatives have two potential advantages. Hydrosoluble derivatives[23,24]

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