Free Rad. Res. Comms., Vol. 7, No. 1, pp. 19-26
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MINIMUM ESSENTIAL STRUCTURAL REQUIREMENTS FOR LACTAM ANTIBIOTIC ACTION

PETER KOVACIC[1]†, JAMES R. AMES[1] and MICHAEL D. RYAN[2]

[1]*Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA*

[2]*Department of Chemistry, Marquette University Milwaukee, WI 53233, USA*

(Received April 11, 1989)

A mechanism of action encompassing mono- and bicyclic β-lactams has been proposed previously, which stresses the importance of formation of an electron transfer (ET) entity (conjugated iminium) as a requirement for antibiotic activity, in association with enzyme inactivation. Additional evidence in support of this contention is now provided. Reduction potentials for several cephalosporins and pyrazolidinones, all of which contain an oximino functionality in the side chain, were observed in the range of −0.6 to −0.7 V. Comparison is made with related compounds lacking imine. Agents containing side chain hydrazone, oxamazins (mono β-lactams), and lactivicin are discussed based on the ET approach.

KEY WORDS: Lactam, antibiotics, mechanism, electron transfer, SAR.

INTRODUCTION

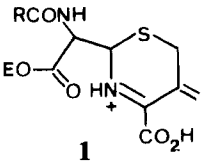
Much attention has been devoted to structure-activity relationships for these antibiotics. The first members of the β-lactam antibiotics discovered were the penicillins and cephalosporins, which possess a fused ring structure. The minimum requirement for activity in these agents is thought to be a β-lactam accompanied by a carboxyl group alpha to the ring.[1,2] About ten years ago, the discovery of the monocyclic antibacterial types, e.g., nocardicins and the monobactams, demonstrated that the fused ring structure was dispensable.[2,3] In more recent times, the reports of active γ-lactams, such as lactivicin, have removed the β-lactam moiety from the essential category.[4] In the large majority of lactam antibiotics, the carboxyl group is present. Exceptions are monobactams which possess a sulphonic acid entity[2] and cephalosporins containing pendant tetrazole.[3] The oxamazins are a further deviation, in which oxygen separates the nucleus from a terminal carboxyl.[5,6] Also important for activity are noncovalent binding parameters (amide side chain, hydrogen bonding, ionic interactions, lipophilicity, hydrophilicity, van der Waals forces, and stereochemistry).

The diverse nature of these antibiotics renders it difficult to identify fundamentally important common features. From our past [7,8] and present investigations, we propose two characteristics that appear to be shared by all members. The first is a lactam ring (β or γ) that effects binding to the bacterium, accompanied by inactivation. The second is a conjugated iminium ion which is generated at the active site and is believed to be responsible for lysis and death via electron transfer (ET).

In relation to the various mechanisms of action of antibacterial agents, interference

†To whom correspondence should be addressed.

with DNA replication or synthesis is one of the most widely accepted routes.[9] Cell membranes and enzymes are also targets.[7,9,10] Recent findings have implicated ET and/or oxidative stress in the activity of synthetic antibacterials[10] and the important β-lactam types.[7,8] On binding to cell wall enzymes, β-lactams, e.g., cephalosporins, are thought to form conjugated iminium species **1** (vide supra) that apparently possess favourable reduction potentials based on studies with model compounds **2**.[7] Bacterial



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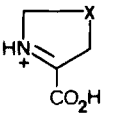


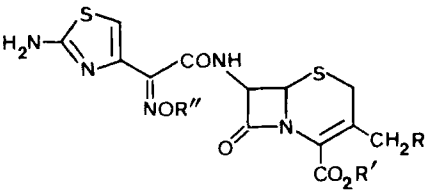
a) X = CH_2
b) X = S

2

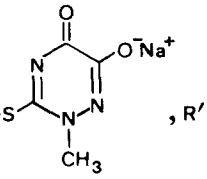
cell death could conceivably result from interference with normal electrophysiological processes by ET reactions involving iminium. The first suggestion of the important participation of electrochemical events was made several decades ago (reviewed in[7]). Thus, site binding not only inactivates enzyme, but also generates a destructive ET entity at a sensitive locus.

Caphalosporin nephrotoxicity provides additional support for this thesis. Oxidative stress resulting from generation of various types of activated oxygen species plays a role;[11–13] antioxidants control the toxicity. Iminium **1** may be responsible.[7]

Recently, the monocyclic types, e.g., nocardicins and monobactams, which are unable to form iminium upon ring opening have been incorporated into the theoretical framework.[8] This class contains functionalities, e.g., oximino, in the side chain that can conceivably act as precursors for ET structures. Furthermore, fused ring β-lactams, such as cephalosporins **3** that also incorporate side chain imine, are generally more potent than those devoid of such entities.[8]



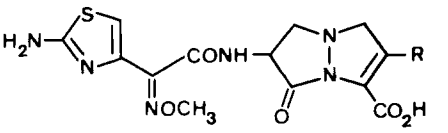
a) R = R′ = H, R″ = CH_3

b) R =  , R′ = Na^+, R″ = CH_3

c) R =  , R″ = $C(CH_3)_2CO_2H$

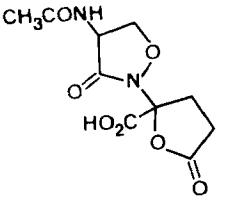
3

Bicyclic pyrazolidinones **4** are a new class of antibacterial agents.[14,15] These compounds potentially contain two ET-generating sites, side chain oxime and γ-lactam nucleus.



4 a) R = CN
b) R = SO_2CH_3

Our objective was to obtain additional information concerning the possible involvement of ET in lactam antibiotic action by examination of the electrochemical characteristics of several cephalosporin types **3** and pyrazolidinones **4**, all of which contain an oximino (imine) functionality in the side chain. Comparison is made with related compounds lacking the imine group. In addition, agents containing a side chain hydrazone, oxamazins (mono-β-lactams), and lactivicin **5** are discussed based on the ET approach.



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MATERIALS AND METHODS

Samples were obained from the following sources: **3a** and **c** and **4a** and **b**, Lilly; **3b**, Hoffmann-La Roche; **7**, Aldrich. Buffer solutions and electrochemical measurements have been previously described.[7,8] The reported values are referenced to the standard hydrogen electrode (SHE).

RESULTS AND DISCUSSION

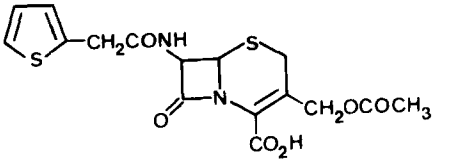
Bicyclic β-Lactams

1. Side Chain Oximes. Our prior report revealed that iminium can commonly be formed from bicyclic β-lactams upon enzyme binding.[7] In some cases, the presence of a side chain oxime can also lead to conjugated iminium. We now report additional examples of this class containing side chain oxime. In electrochemical studies, compound **3a** reduced at -0.61 V at pH 4.1 (Table I). The process ($E_{pp/2}$ = 85 mV) contained no oxidation current at the sweep rate examined. A plot of E_p versus log of the scan rate gave a slope of 35 mV, characteristic of follow-up chemistry subsequent to electron transfer (EC mechanism). The potential is a result of the oximino reduction c.f. **6**, $E_p > -1.0$ V.[8]

TABLE I
Cyclic Voltammetry of Conjugated Imines and 7[a]

Compound	$-E_p$(V)	$E_{pp/2}$(mV)
3a	0.61	85
3b	0.58[b]	55
3c	0.64, 0.98	165
4a	0.72[c]	100
4b	0.70[c]	115
7	0.96	80
Benzil monohydrazone	0.26[d]	–
Benzil monoxime	0.40[d]	–
Pyruvic acid oxime		
syn	0.18[e]	–
anti	0.13[e]	–

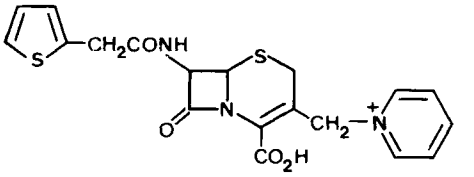
[a] 100 mV/s, compound 0.5 mM, HMDE versus NHE, pH 4.1 unless otherwise indicated.
[b] 5% DMF/EtOH (60/40)/pH 3.6.
[c] 10% DMF.
[d] References 17 and 18, pH 4.
[e] Reference 27, pH 1.



6

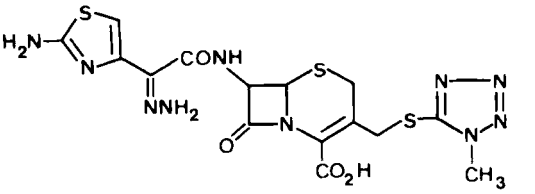
Ceftriaxone **3b** reacted at -0.58 V (pH 3.6) in an irreversible manner. However, an $E_{pp/2}$ value of 55 mV indicates potential reversibility. A plot of i_{pc} versus (scan rate)$^{1/2}$ ($r = 0.998$) passed through the origin. These results imply an EC mechanism.

Compound **3c**, ceftazidime, reduced at values slightly less than **3a** and **b**: $E_p = -0.64$ V with $E_{pp/2}$ of 165 mV, which may be due to presence of the carboxyl group. Structurally related antibacterials behave similarly.[8] A second wave at -0.99 V results from the pyridinium moiety; compare cephaloridine **7** with E_p of -0.96 V. Participation of the pyridinium moiety in ET, as previous proposed,[11] is unlikely due to the unfavourable potential. Thus, iminium generated from the side chain or from enzyme acylation (see **1**) might be responsible for some of the cytotoxic manifestations (*vide supra*).



7

2. Side Chain Hydrazones Very little has been reported on the unsubstituted or substituted 2-(2-aminothiazol-4-yl)–2-hydrazono cephalosporins, e.g., **8**.[16] Activity is slightly lower than for cefuroxime and cefotaxime. Since hydrazone derivatives of carbonyls reduce at potentials similar to the corresponding oximes[17,18] (Table I), the ET characteristics should correspond closely.

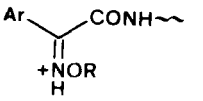


8

A number of correlations between antibacterial activity and reduction potential can be made in the cephalosporin series. Cefuroxime ($E_p = -0.44$ V)[8] is more active[19,20] than **3c**. Ceftriaxone (**3b**) is comparable[21–24] to, or more active than, cefotaxime ($E_p = -0.71$ V)[8] which is about equal in potency to **3c**.[25] Other relationships have been noted, but there are also exceptions.[8]

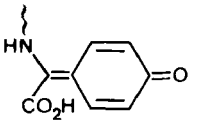
Monocyclic β-Lactams

Since the monocyclic ring cannot generate conjugated iminium upon ring opening with enzyme, apparently side chain imine assumes the ET function for this class. In our prior studies[8] a significant increase in E_p was found upon protonation to form **9**. For example, in the case of the monobactams, E_p was about -0.6 at pH 4.1; similarly, nocardicin A reacted at -0.47 V. Previously, we discussed the possible ET involve-



9

ment of the labile quinonemethide **10** which is derived *in vivo* from nocardicin.[8] This metabolite may also form an iminium (**11**) in conjugation with carboxyl (*vide supra*) by tautomerization.



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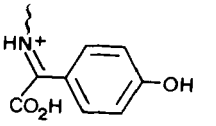
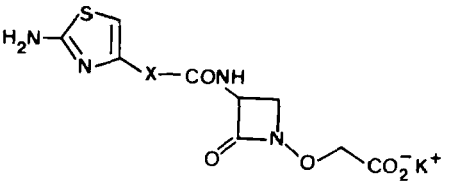


11

A more recent class, the oxazamins **12**, has shown activity.[5,6] It is significant that oxime **12a** possesses higher activity compared to **12b**, devoid of the imine group.



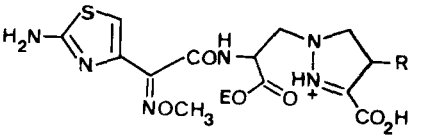
12 a) X = C = NOCH$_3$

b) X = CH$_2$

Based on prior electrochemical studies with related types,[8] **12a** in protonated from would be expected to exhibit ET properties.

γ-Lactams

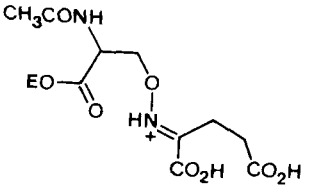
1. Pyrazolidinones.[14,15] These γ-lactams are capable of forming an ET entity (iminium carboxylic acid) (**13**), analogous to **1**, upon acylation of the cell wall enzyme (EOH).



13

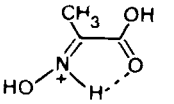
In this study we examined the redox characteristics of the side chain oxime (Table I). Compound **4a** reduced in a diffusion controlled manner at -0.72 V. Irreversibility was indicated by the absence of any reoxidation current and a 100 mV difference for $E_p - E_{p/2}$. Antibiotic **4b** behaved similarly to the cyano analog: $E_p = -0.70$ V, $E_{pp/2} = 115$ mV. A second peak was observed at -1.01 V.

2. Lactivicin. Lactivicin **5** possesses antibiotic activity.[4,26] Biological studies suggest a mode of action similar to β-lactams.[4] It is significant that **5** appears capable of forming conjugated iminium **14** on binding with enzyme (EOH).



14

As in the β-lactam types, carboxyl α to the lactam N is important; L-cycloserine and the N-acetyl derivative do not exhibit antibacterial activity.[4] Electrochemical studies demonstrate a large, favorable influence of carboxyl on iminium reduction potential.[7] The imine carboxyl structure is electronically akin to the diimine portion of flavins. Stereochemistry (*syn* or *anti*) at the active site may influence electrochemistry, e.g., the $E_{1/2}$(pH 1)[27] values for oximes of pyruvic acid (simple models of **14**) are given in Table I. The protonated anti isomer **15** (more positive potential) can form an intramolecular hydrogen bond analogous to iminium **2**, which would stabilize the ionic state.



15

Several other γ-lactams, related to **6** and **7**, exhibit low levels of antibiotic activity.[28–30]

Quinlan and Gutteridge showed that β-lactam antibiotics in the presence of certain metal ions mimic phagocytic cells by generating activated oxygen entities.[31] A naturally occurring mono β-lactone antibiotic possesses two potentially electroactive sites, namely, a nitroarene and catechol (metal chelator and o-quinone precursor).[32]

The captodative effect[33,34] may be of importance in the ET characteristics of iminium species derived from ring opening or from the side chain. The imine carboxyl functionality formed during ring opening is also present in simple related compounds, e.g. **2a**, which are known to possess physiological activity that may be associated with ET.[33]

Most of the reactions in this study were irreversible. Factors affecting reversibility and magnitude of the potential *in vivo* have been discussed.[35,36]

References to other xenobiotics (carcinogens, benzodiazepines, anticancer agents, bactericides, antimalarials, mesoionics, spermine, phencyclidine, nicotine, amoebicides, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, antiprotozoan agents, and cocain) that may act via ET are provided elsewhere.[8] More recent studies include anthelmintics[37] and anticancer agents.[38,39]

In summary, essentially all of the lactam antibiotics can be mechanistically accommodated within the dual concept of enzyme acylation and generation of a conjugated iminium moiety. Perhaps, delineation of the crucial structure-activity relationships is not an "impossible dream".[26]

Acnowldegements

The authors thank Lilly (Drs. L.N. Jungheim and T.K. Hatfield). Hoffmann-LaRoche, and Professor T. Fukuyama (Rice University) for assistance.

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Accepted by Prof. J.M.C. Gutteridge