Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 193–196

BIOORGANIC & MEDICINAL CHEMISTRY

N-Thiolated β-Lactam Antibacterials: Defining the Role of Unsaturation in the C_4 Side Chain

Cristina Coates,[a] Timothy E. Long,[a] Edward Turos,[a,*] Sonja Dickey[b] and Daniel V. Lim[b]

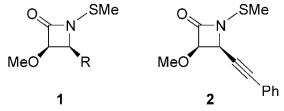
[a]*Department of Chemistry, University of South Florida, Tampa, FL 33620, USA*
[b]*Department of Biology, University of South Florida, Tampa, FL 33620, USA*

Received 10 June 2002; accepted 7 August 2002

Abstract—*N*-Methylthio β-lactams represent a novel family of antibacterial agents for methicillin-resistant *Staphylococcus aureus* (*MRSA*). The structure–activity functions and mechanism of action of these compounds, although still largely undefined, differ dramatically from those of all previously reported β-lactam antibiotics. Prior work has established that the *N*-alkylthio moiety is required for antibacterial activity, and that a variety of unsaturated groups can be tolerated at C_4 of the lactam ring. This report describes the effect that unsaturation within the C_4 substituent has on antibacterial activity of these interesting new *N*-thiolated β-lactams.

Introduction

N-Thiolated β-lactams (**1**) are a new family of antibacterial compounds that selectively inhibit the growth of *Staphylococcus* species, including methicillin-resistant *Staphylococcus aureus* (*MRSA*).[1] Lead compound **2** was identified in our studies on bicyclic and monocyclic *N*-thiolated β-lactams, prompting us to explore the structural features which give the molecule its antimicrobial properties.[2,3]



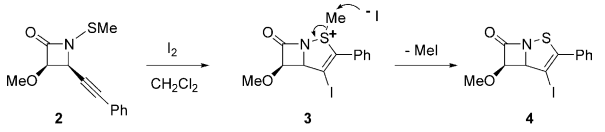
What makes the discovery of these antibacterial properties for lactam **2** so intriguing is the fact that the compound possesses only lipophilic side chains on the four-membered ring, rather than the ionic or acidic functionality that other β-lactam drugs require for binding to their transpeptidase targets. Microscopy studies revealed that lactam **2** does not cause the morphological defects in bacterial cells expected of an inhibitor of cell wall synthesis such as penicillin.[4] The high selectivity for *Staphylococcus* over most other common bacteria tested, and the ability to maintain its antibacterial activity against β-lactamase-producing strains of *MRSA*, are highly unusual. The structure–activity parameters and mechanism of action, although still ill-defined, clearly differ from those of traditional β-lactam drugs: the *N*-methylthio group is essential for activity, while the alkynyl substituent at C_4 can be replaced with an alkenyl, aryl or heteroaryl group without loss of antimicrobial capabilities.[5] However, whether these substituents play a specific role in the antibacterial activity has not yet been determined. In this report, we address one of the first questions pertaining to this: the need for unsaturation within the C_4 side chain.

Results and Discussion

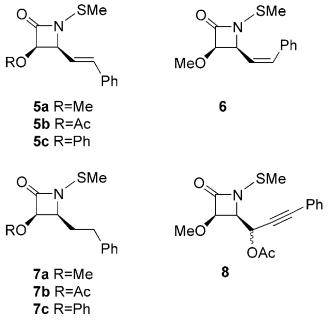
Our initial motivation to examine the role of the C_4 substituent in the antibacterial properties of these *N*-thiolated lactams came from prior work in our laboratory, which showed that monocyclic lactam **2** can ring close to isopenem **4** in the presence of molecular iodine.[2,3] The reaction produces a powerful alkylating species, bicyclic intermediate **3**, which undergoes *S*-demethylation to afford **4**. We questioned whether a similar process, triggered possibly by a biological electrophile, could be occurring in vitro to account for the biological activity of lactam **2**.

*Corresponding author. Tel.: +1-813-974-7312; fax: +1-813-974-1733; e-mail: eturos@chuma.cas.usf.edu

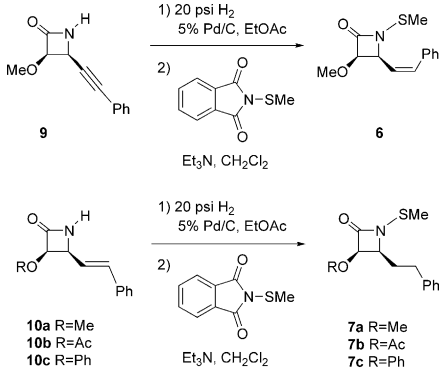
0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00383-8



To investigate this, a series of *N*-methylthio lactams **5**–**8** were synthesized and their antimicrobial activities evaluated. β-Lactams **5** and **8** were prepared as previously described from the acid chlorides and *N*-(4-methoxyphenyl)aryl imine.[2] *Z*-Alkenyl derivative **6** was synthesized by catalytic hydrogenation of the alkynyl precursor **9**, followed by *N*-methylthiolation with *N*-(methylthio)phthalimide.[6] Similarly, the C_4-phenylethyl analogues **7** were produced in two steps from the *E*-alkenyl lactams **10**.



β-Lactams **5**–**8** were screened for antibacterial activity against a panel of methicillin-susceptible and methicillin-resistant *Staphylococcus* species, using a standard Kirby–Bauer disk diffusion protocol. The zones of growth inhibition obtained for each lactam and microbe are given in Table 1. The data indicates that although the *E*-lactam **5a** has good activity against *S. aureus*, it is less effective than alkynyl compound **2**. The same is true for the *MRSA* isolates. Within the *E*-alkenyl series, the C_3-methoxy lactam **5a** and C_3-acetoxy compound **5b** are slightly more active than the C_3-phenoxy derivative **5c** on an equal weight basis (20 μg/disk). The compounds express good activity against other *Staphylococcus* species, including *S. simulans, S. saprophyticus*, and *S. epidermidis* as well as *Micrococcus luteus*.



The fact that these three alkenyl compounds have lower activity than the alkynyl analogue **2** may, curiously enough, provide support for an electrophile-initiated cyclization-dealkylation process depicted above. Solution experiments have found that the structurally related *E*-alkenyl lactam **11** undergoes ring closure in the presence of iodine much more slowly than alkynyl compound **2**. This may be due to conformational or stereoelectronic nuances of the cyclization. It is also interesting that the *Z*-compound **6** produces larger inhibition zones than the *E*-isomer **5a**, indicating that olefin geometry in the C_4 side chain can be a contributing factor. This is also consistent with a cyclization step, based on our earlier report that 4-butynyl benzyl sulfide undergoes iodocyclization faster than the *Z*-4-butenyl sulfide, which in turn cyclizes more rapidly than its *E*-isomer.[3] Thus, all the data for these C_4 unsaturated analogues is suggestive of a cyclization-dealkylation mechanism.

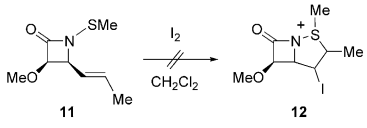


Table 1. Growth inhibition zones obtained from agar disk diffusion experiments using 6-mm air-dried disks impregnated with 20 μg of the test compound. The values are the averages of three runs. The values correspond to the average diameters in mm for the zone of growth inhibition observed after 24 h. *Staphylococcus aureus* and β-lactamase-producing strains of methicillin-resistant *Staphylococcus aureus* (labeled *MRSA* USF652–659) were obtained from a clinical testing laboratory at Lakeland Regional Medical Center, Lakeland, FL. ('nt' indicates 'not tested')

Microorganism	2	5a	5b	5c	6	7a	7b	7c	8	Penicillin G
Staphylococcus aureus ATCC 25923	27	17	14	12	21	17	17	14	17	34
MRSA USF652	29	18	12	14	25	18	17	14	19	8
MRSA USF653	29	22	15	13	26	20	18	12	21	16
MRSA USF654	27	16	14	12	22	17	17	13	18	10
MRSA USF655	27	17	14	12	21	15	15	11	18	14
MRSA USF656	30	17	15	13	24	17	18	13	19	12
MRSA USF657	28	16	14	13	22	14	17	12	17	12
MRSA USF658	27	15	13	12	21	15	15	12	17	19
MRSA USF659	24	14	10	10	17	15	16	12	16	15
Staphylococcus simulans	19	11	8	7	nt	16	13	8	13	13
Staphylococcus saprophyticus	23	12	10	9	nt	11	11	10	15	30
Staphylococcus epidermidis	30	23	20	14	nt	18	18	12	24	50
Micrococcus luteus	28	20	14	15	nt	23	23	17	24	40

No zones of growth inhibition were observed for the following bacteria: *Bacteroides fragalis* (clinical isolate), *Enterobacter cloacae* (environmental isolate), *Escherichia coli* (ATCC 23590), *Klebsiella pneumoniae* (environmental isolate), *Neisseria gonorrhoeae* (clinical isolate, β-lactamase positive), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella typhimurium* (clinical isolate), *Serratia marcescens* (ATCC 29634), *Vibrio cholerae* (biotype E1 Tor Ogawa, cholera toxin positive, CDC E5906).

We then examined the C_4 phenylethyl lactams **7a–c**, which on the basis of the above mechanistic consideration would be devoid of antibacterial capabilities since the alkene or alkyne functionality is absent. However, *all three of these derivatives proved to be more active than their E-alkenyl counterparts*. Thus, unsaturation at the α carbon of the C_4 side chain is clearly *not* a requirement for antimicrobial activity. This is further illustrated in the case of the propargylic derivative **8**, which also shows good antibacterial activity despite having its unsaturation at the β-carbon of the side chain. Other types of conformationally flexible groups and functionalities may also be effective.

Conclusion

These findings have enabled us to determine that the antimicrobial activity of *N*-methylthio β-lactams is not associated strictly with the presence of unsaturation on the C_4 side chain. Thus, the fact that saturated C_4 substituents are also acceptable groups rules out the involvement of an electrophile-initiated cyclization-dealkylation process. However, this does not exclude the possibility that a biological nucleophile could attack the *S*-methyl carbon center directly in an S_N2 fashion.[7] β-Lactam antibiotics generally react with biological nucleophiles at the carbonyl with cleavage of the four-membered ring. However, for these *N*-thiolated β-lactams, thiophilic[8] reagents attack the sulfur center to cause N–S bond cleavage. Whether this mode of reactivity is responsible for the biological properties of these molecules is the subject of our current investigations.

Experimental

All reagents needed for the synthesis of the β-lactams were purchased from Sigma-Aldrich Chemical Company and used without further purification. Solvents were obtained from Fisher Scientific Company. Thin layer chromatography (TLC) was carried out using EM Reagent plates with a fluorescence indicator (SiO_2-60, F-254). NMR spectra were run in $CDCl_3$. Products were purified by flash chromatography using J. T. Baker flash chromatography silica gel (40 μm). β-Lactams **2** and **8** are previously reported compounds.[2]

Lactams **5a–c** were prepared from their commercially available aldehydes using the procedures described in ref 3.

(5a). White solid, mp 92–94 °C, 81% yield. IR (cm^{-1}) 1755 (β-lactam C=O); ^{1}NMR (250 MHz) δ 7.47–7.30 (m, 5H), 6.81 (d, J = 15.9 Hz, 1H), 6.25 (dd, J = 15.9, 9.3 Hz, 1H), 4.71 (d, J = 4.8 Hz, 1H), 4.40 (dd, J = 9.3, 4.8 Hz, 1H), 3.48 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (63 MHz) δ170.1, 137.4, 135.8, 128.6, 128.4, 126.7, 122.3, 86.1, 65.6, 58.7, 22.8.

(5b). White solid, mp 128–133 °C, 45% yield. IR (cm^{-1}) 1760 (β-lactam C=O), 1741; ^{1}NMR (250 MHz) δ 7.42–7.30, (m, 5H), 6.77 (d, J = 15.9 Hz, 1H), 6.06 (dd, J = 15.9, 8.9 Hz, 1H), 5.91 (d, J = 5.4 Hz, 1H), 4.57 (dd, J = 8.8, 5.0 Hz, 1H), 2.48 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (63 MHz) δ168.9, 168.1, 138.2, 135.5, 128.7, 128.6, 126.6, 120.7, 64.9, 22.7, 20.2.

(5c). White solid, mp 103–110 °C, 65% yield. IR (cm^{-1}) 1765 (β-lactam C=O); ^{1}NMR (250 MHz) δ 7.38–7.22, (m, 7H), 6.98 (dd, J = 4.7, 2.6 Hz, 3H), 6.81 (d, J = 15.8 Hz, 1H), 6.26 (dd, J = 15.8, 9.2 Hz, 1H), 5.44 (d, J = 4.9 Hz, 1H), 4.60 (dd, J = 9.2, 4.9 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (63 MHz) δ168.9, 157.0, 137.9, 135.8, 129.5, 128.6, 128.5, 126.8, 122.4, 121.8, 115.5, 82.8, 65.8, 22.9.

Synthesis of lactam 6. A solution of **9a** (93 mg, 0.46 mmol) in EtOAc (10 mL) containing a catalytic amount of 5% Pd on carbon was hydrogenated under 20 psi H_2 for three h. The solvent was removed under reduced pressure to yield a yellow oil. The crude product was separated by flash chromatography to yield 17 mg (18% yield) of a light brown oil. ^{1}NMR (250 MHz) δ 7.40–7.19 (m, 5H), 6.81 (d, J = 11.6 Hz, 1H), 6.28 (br s, 1H), 5.81 (dd, J = 11.5, 8.8 Hz, 1H), 4.68 (m, 2H), 3.55 (s, 3H). ^{13}C NMR (63 MHz) δ 167.9, 136.1, 134.8, 128.53, 128.48, 127.7, 127.4, 86.8, 58.8, 52.3.

To a solution of the above product (17 mg, 0.085 mmol) in CH_2Cl_2 (2 mL) was added *N*-(methylthio)phthalimide (16 mg, 0.085 mmol) and a few drops of triethylamine. The mixture was refluxed overnight. Upon completion of the reaction, the solvent was removed under reduced pressure to yield a brown oil. The crude material was purified by column chromatography on silica gel to yield **6** as a colorless oil, 11 mg in 53% yield. ^{1}NMR (250 MHz) δ 7.38 (s, 1H), 6.99 (d, J = 11.6 Hz, 1H), 5.80 (dd, J = 11.1, 10.3 Hz, 1H), 4.74 (dd, J = 9.9, 4.9 Hz, 1H), 4.64 (d, J = 4.9 Hz, 1H), 3.52 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (63 MHz) δ 173.0, 140.1, 131.4, 131.2, 130.6, 127.8, 89.1, 62.9, 61.7, 25.6.

Similar conditions as above were used to synthesize lactams **7a–c** from **10a–c**, intermediates prepared in the synthesis of **5a–c**.

(7a). Yellow oil, 50% yield. IR (cm^{-1}) 1760 (β-lactam C=O); ^{1}NMR (250 MHz) δ 7.34–7.21 (m, 5H), 4.54 (d, J = 5.1 Hz, 1H), 3.78 (dd, J = 12.7, 5.8 Hz, 1H), 3.57 (s, 3H), 2.79 (t, J = 6.9 Hz, 2H), 2.44 (s, 3H), 2.15–1.97 (m, 2H). ^{13}C NMR (63 MHz) δ 171.1, 141.1, 128.4, 128.3, 126.0, 84.8, 61.5, 59.1, 31.8, 30.2, 22.6.

(7b). Beige solid, mp 68–71 °C, 56% yield. IR (cm^{-1}) 1765 (β-lactam C=O), 1764; ^{1}NMR (250 MHz) δ 7.33–7.17 (m, 5H), 5.89 (d, J = 4.8 Hz, 1H), 3.90 (dd, J = 11.4, 5.6 Hz, 1H), 2.84–2.61 (m, 2H), 2.47 (s, 3H), 2.13 (s, 3H), 2.08 (overlapping m, 1H), 1.94 (m, 1H). ^{13}C NMR (63 MHz) δ 169.3, 168.4, 140.5, 128.6, 128.2, 126.3, 76.0, 61.2, 31.8, 30.3, 22.6, 20.4.

(7c). Beige solid, mp 59–61 °C, 61% yield. IR (cm^{-1}) 1760 (β-lactam C=O); ^{1}NMR (250 MHz) δ7.37–7.02 (m, 10H), 5.36 (d, $J = 5.1$ Hz, 1H), 4.00 (app q, $J = 5.5$ Hz, 1H), 2.84 (t, $J = 8.0$ Hz, 2H), 2.52 (s, 3H), 2.22 (m, 2H). ^{13}C NMR (63 MHz) δ 169.4, 157.4, 140.9, 129.6, 128.5, 128.3, 126.1, 122.3, 115.5, 81.5, 61.7, 31.9, 30.5, 22.7.

Testing of antimicrobial susceptibilities (Kirby–Bauer disk diffusion)

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 23590), *Serratia marcescens* (ATCC 29634), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus saprophyticus* (ATCC 3552), *Staphylococcus simulans* (ATCC 11631) were purchased from ATCC sources. *Vibrio cholerae* (biotype E1 Tor Ogawa, cholera toxin positive, CDC E5906) was obtained from the Centers for Disease Control in Atlanta, GA. The following environmental isolates were obtained from Lakeland Regional Medical Center, Lakeland, FL: *Staphylococcus aureus* (methicillin-resistant, β-lactamase-producing, labeled as *MRSA* USF652–659 in Table 1), *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. *Bacteroides fragalis* was obtained from Smith-Kline Laboratories. *Neisseria gonorrhoeae* (β-lactamase positive) was obtained from the Tampa Branch State Laboratory.

Culture preparation. From a freezer stock in tryptic soy broth (Difco Laboratories, Detroit, MI) and 20% glycerol, a culture of each microorganism was transferred with a sterile Dacron swab to Trypticase® Soy Agar (TSA) plates (Becton-Dickinson Laboratories, Cockeysville, MD), streaked for isolation and incubated at 37 °C for 24 h. A 10^8 standardized cell count suspension was then made in sterile phosphate buffered saline (pH 7.2) and swabbed across fresh TSA plates.

Disc preparation. Sterile 6-mm susceptibility discs (Becton-Dickinson Laboratories, Cockeysville, MD) were impregnated with 20 μL of a 1 mg/mL stock solution of the test lactam compound in dimethylsulfoxide (DMSO) and placed onto the inoculated TSA plates. The plates were incubated for 24 h at 37 °C and the antimicrobial susceptibilities were determined by measuring the zones of growth inhibition around each disc.

Determination of minimum inhibitory concentrations

Media preparation. The minimum inhibitory concentrations (MIC) values of the lactams were determined for *Staphylococcus aureus* and *MRSA* by agar dilution. The test medium was prepared in 24 well plates (Costar 3524, Cambridge, MA) by adding a known quantity of the test drug in DMSO to Mueller–Hinton II agar (Becton-Dickinson Laboratories, Cockeysville, MD) to bring the total volume in each well to 1.0 mL. The medium was allowed to solidify at room temperature for 24 h before inoculation with the bacteria. Using a sterilized inoculating loop, a small amount of each standardized *Staphylococcus* strain cultured on TSA plates for 24 h was transferred into sterile test tubes containing 5 mL of TSA broth, and incubated at 37 °C for 24 h. One microliter of each culture was then applied to the appropriate well of Mueller-Hinton agar and incubated at 37 °C overnight. After 24 h, the MICs were determined as being the lowest concentration of drug in the agar where no bacterial growth was visible. MIC values for lactams **5**–**8** were around 20 μg/mL.

Acknowledgements

Funding for this research was generously provided by the National Institutes of Health (R01 AI51351).

References and Notes

1. Turos, E.; Konaklieva, M. I.; Ren, R. X. F.; Shi, H.; Gonzalez, J.; Dickey, S.; Lim, D. *Tetrahedron* **2000**, *56*, 5571.
2. Ren, X.-F.; Konaklieva, M. I.; Shi, H.; Dickey, S.; Lim, D. V.; Gonzalez, J.; Turos, E. *J. Org. Chem.* **1998**, *63*, 8898.
3. Ren, X.-F.; Konaklieva, M. I.; Turos, E. *J. Org. Chem.* **1995**, *60*, 4980.
4. *Chemistry and Biology of β-Lactam Antibiotics* ; Morin, R. B., Gorman, M., Eds., Academic Press: New York, 1982, Volumes 1–3.
5. Turos, E.; Long, T. E.; Konaklieva, M. I.; Coates, C.; Shim, J.-Y.; Dickey, S.; Lim, D. V.; Cannons, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2229.
6. Woulfe, S. R.; Iwagami, H.; Miller, M. J. *Tetrahedron Lett.* **1985**, *26*, 3891.
7. This postulated *S*-demethylation would resemble that which occurs in vivo in the transfer of *S*-adenosylmethionine to nitrogen bionucleophiles.
8. Shah, N. V.; Cama, L. D. *Heterocycles* **1987**, *25*, 221. Likewise, we have observed in solution studies that the *N*-methylthio lactams react with glutathione via transfer of the methylthio group with N–S bond cleavage.