Bioorganic & Medicinal Chemistry Letters, Vol.3, No.11, pp. 2241-2246, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

N-ACYL 3-ALKYLIDENYL- AND 3-ALKYL AZETIDIN-2-ONES: A NEW CLASS OF MONOCYCLIC β-LACTAM ANTIBACTERIAL AGENTS 2. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF HETEROATOM SUBSTITUTED 3-ISOPROPYLIDENE AND 3-ISOPROPYL ANALOGS

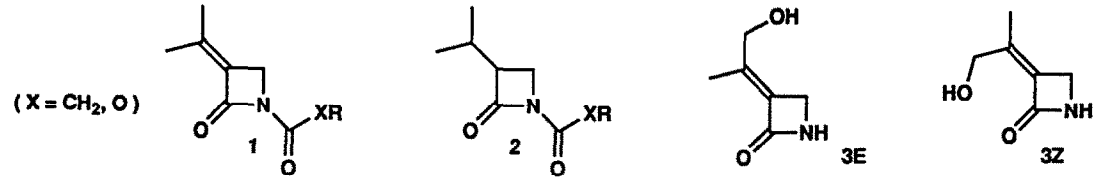
Steven J. Brickner,* Jeffrey J. Gaikema, Laura J. Greenfield,
Gary E. Zurenko and Peter R. Manninen

Upjohn Laboratories
The Upjohn Company, Kalamazoo, MI 49001, USA

(*Received* 1 *April* 1993)

Abstract: O-, N-, or F-substituted 3-isopropylidene- and 3-isopropyl N-acyl azetidin-2-ones, lacking an ionizable moiety attached to the lactam nitrogen, have *in vitro* antibacterial activity, being particularly potent *vs* anaerobes.

3-Isopropylidenyl-(**1**) and 3-isopropyl (**2**) N-acyl azetidin-2-ones are novel synthetic monocyclic β-lactams exhibiting *in vitro* antibacterial activity *vs* anaerobes and Gram-positive aerobes.[1] A unique structural aspect differentiating azetidinones **1** and **2** from all other classes of β-lactam antibiotics is the lack of any negatively-charged group (eg., $CHRCO_2^-$ or SO_3^-) appended to the lactam nitrogen. Such anionic appendages are generally considered essential structural components, believed to play a role in attachment of substrate to the transpeptidases involved in bacterial cell wall biosynthesis.[2] A report that the related N-acyl 3-alkyl *4-acetoxy* azetidin-2-ones inhibit human leukocyte elastase[3] indicates a broadening interest in 3-alkyl N-acyl azetidinones.

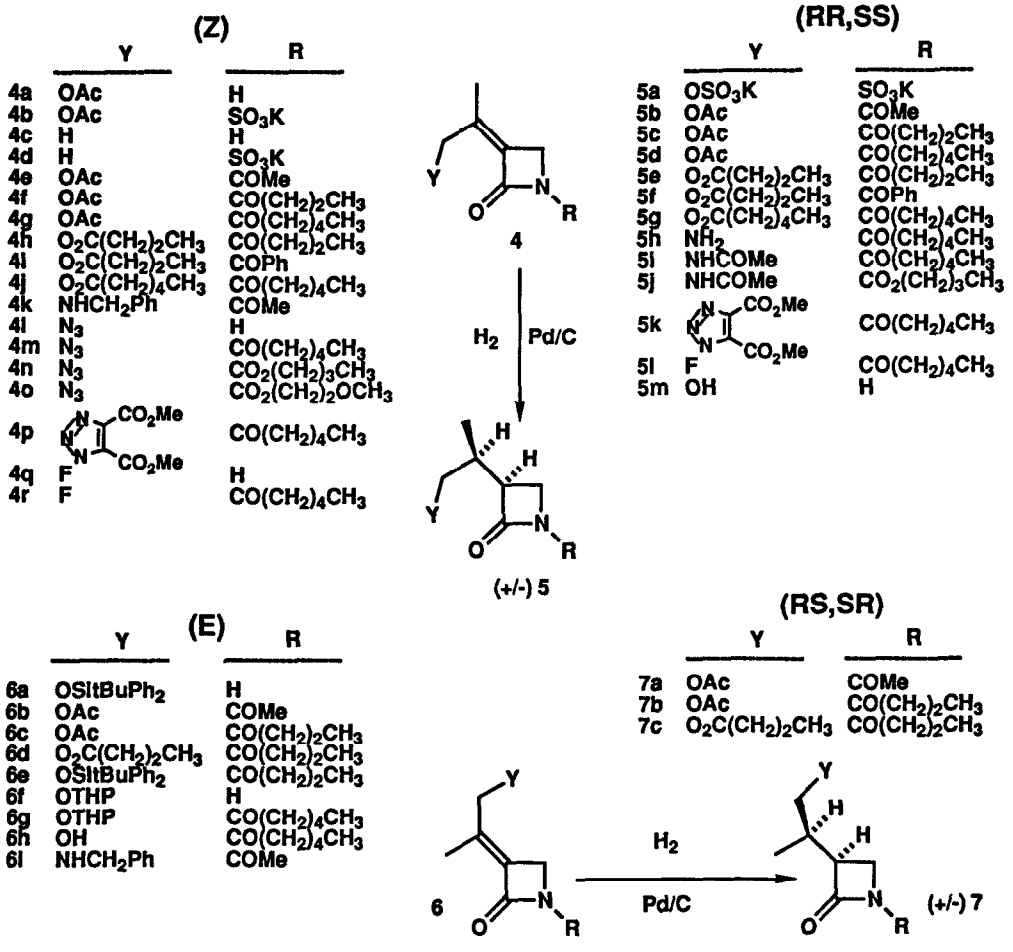


At the conceptual stage in our lead-finding program to examine the utility of N-1 activated 3-alkylidene azetidin-2-ones as potential antimicrobial agents, we required a template with a suitable synthetic handle, allowing for functionalization about the β-lactam. The ability to readily introduce a wide variety of side-chains or functionalities was a desirable feature, allowing us to maximize the chances of finding structural attributes which would enhance delivery to, or binding of, the β-lactam at the targeted transpeptidase active site.[4] In this regard, the 3-(hydroxyisopropylidene)azetidin-2-ones **3Z** and **3E** were synthesized.[5] Herein we report the synthesis and

antibacterial activity of various heteroatom-substituted 3-isopropylidenyl- and 3-isopropyl N-acyl azetidin-2-ones, derived from these key intermediates.

The N-1 group we had initially selected for electronic activation of template **3** was the sulfonate.[6] Treatment of E-siloxyisopropylidene azetidinone **6a**[5] with DMF-SO_3 resulted in silyl cleavage and resultant O,N-bis-sulfonation. Alternatively, the **Z** allylic acetate **4a** was prepared from **3Z**, and N-sulfonation followed by K^+ ion exchange provided N-sulfonate **4b**. This, and the similarly-derived **4d** (from **4c**[5]), as well as the O,N-bis-sulfonated isopropyl compound **5a** (from **5m**, the hydrogenation product of **3Z**), were devoid of antibacterial activity.

The lead N-acyl azetidinone **4e** originated as a minor by-product isolated during the preparation of **4a**. As the β-lactam carbonyl IR absorption (ν = 1781 cm^{-1}) of this N-acetyl derivative was indicative of a highly-activated, electrophilic azetidinone carbonyl,[7] **4e** was submitted for broad-spectrum antibacterial screening using



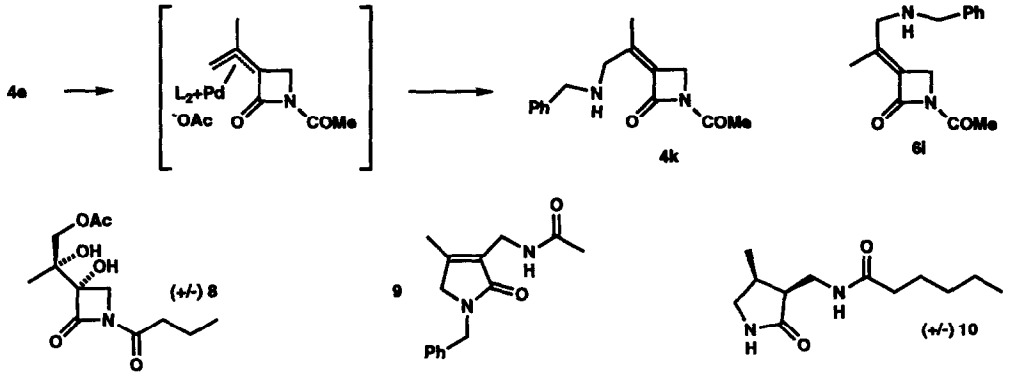
a standard agar diffusion assay. Surprisingly, this compound exhibited activity (29 mm zone, 80 μg/12.8 mm disk), *specifically* against the Gram-negative anaerobe, *Bacteroides fragilis*.

Systematic variation of the O- and N-acyl side-chain lengths was carried out in both the E (**6b-d**) and Z (**4e-j**) series. Mixed O,N-bisacyl analogs were derived by selectively monoacylating on the hydroxyisopropylidene moiety {$(RCO)_2O$, pyridine}, followed by N,N-dimethylaminopyridine (DMAP)-catalyzed anhydride acylation at N-1, or treatment with an acid chloride or chloroformate and Et_3N.

Catalytic hydrogenation (1 atm H_2, Pd/C, EtOAc) of the Z isopropylidene analogs **4** produced the corresponding (+/-) (*RR,SS*)-dihydro derivatives **5** in high yields; reduction of E olefins **6** similarly yielded the diastereomeric (+/-) (*RS,SR*) 3-(substituted-isopropropyl) N-acyl azetidin-2-ones **7**. Stoichiometric osmylation in pyridine of the E-isopropylidene **6c** gave diol **8** (98%).

Most of our SAR studies concentrated on the **4** (Z) and **5** (*RR,SS*) series, given that they were found more active than the isomeric congeners (*vide infra*). However, one N-acyl E isopropylidene azetidinone was of particular interest, in that it shared the α-(hydroxyisopropylidene) β-lactam nucleus of the carbapenem dihydroasparenomycin.[8] Attempted synthesis by exposure of the N-butyrylated silylether **6e** to n-Bu_4NF resulted in decomposition of the β-lactam. Therefore, alcohol **3E** was capped as the tetrahydropyranyl (THP) ether **6f**, then N-hexanoylated to give **6g**. While THP removal with p-toluene sulfonic acid in MeOH was successful, with tlc monitoring of the reaction course indicating a one-spot conversion, recovery of the crystalline **6h** was only 24% after evaporative workup and flash chromatography (perhaps due to inherent instability).

Compound **4e**, a γ-oxo allylic acetate, presented the opportunity to examine the potential use of such substrates for selective palladium-catalyzed π-allyl alkylations, in the presence of a highly-activated carbonyl electrophile.[9] Treatment of **4e** with 1.5 equiv of benzylamine {0.2 equiv $Pd(P\Phi_3)_4$ and $P\Phi_3$, THF, 20°C, 42 h) indeed gave the isomeric[10] amines **4k** (16%) and **6i** (18%). At THF reflux temperature (4.5 h), a complex mixture was obtained, from which were isolated γ-lactam **9** (51%), **6i** (11%), and only traces of **4k**.



Mesylation of **3Z** (Et_3N, MsCl, THF, 0°C), followed by displacement with NaN_3, afforded crystalline azide **4l** (98% from **3Z**). DMAP-catalyzed N-hexanoylation gave **4m**, treatment with BuOCOCl provided **4n**, and methoxyethyl chloroformate gave **4o**. Not unexpectedly, hydrogenation of **4m** (10% Pd/C, 1 atm H_2, EtOAc) resulted in concomitant attack of the nascent amine on the β-lactam carbonyl, yielding γ-lactam **10** (90%). Intramolecular attack was averted by running the reduction in neat Ac_2O, effectively trapping the isopropylamine moiety in **5h** as the acetamide **5i** (93%); similarly, ester-activated lactam **4n** gave **5j**. 1,3-Dipolar cycloaddition of **4m** and dimethylacetylene dicarboxylate gave 1,2,3-triazole **4p** (20°C, 1 week, 100%), which was hydrogenated to **5k**.

Treatment of **3Z** with diethylaminosulfurtrifluoride (CH_2Cl_2, -30°C) provided fluoride **4q** (16%, unoptimized). N-Hexanoylation gave the fluoroisopropylidene compound **4r**; hydrogenation gave **5l** (with a small amount of fluoride hydrogenolysis).[11]

Biological Activity

Table I gives Minimal Inhibitory Concentration (MIC) values for selected organisms, and Table II gives zones of inhibition for anaerobes and Gram-positive aerobes. Unlike the lead **Z**-configured **4e**, **6b** and others esters in the **E** series, while active against both Gram-negative and Gram-positive anaerobes, were generally weaker in activity. Among the **Z** O,N-bisacyl compounds, the O-acetyl N-hexanoyl analog **4g** was most active. Azide **4m** and fluoride **4r** exhibited a similar spectrum of activity (anaerobes and Gram-positives, including multiply-antibiotic resistant[12] *Staphylococcus aureus* UC®6685), compared to the unsubstituted parent **1**[1] {X=CH_2, R=$(CH_2)_3CH_3$}. Some of these esters were active against *Mycobacterium avium*. Benzylamines **4k** and **6i**, and

TABLE I. MINIMAL INHIBITORY CONCENTRATIONS (μg/mL)

ORGANISMS	UC #	2a	5b	5c	5e	5g	7c	Az	Cl
Bacteroides thetaiotaomicron	6360	1†	128	64	32	8	128	>256	4
Bacteroides melaninogenicus	6523	NT	128	64	8	8	64	4	<0.12
Bacteroides fragilis	6428	2††	256	>128	64	8	256	128	<0.12
Bacteroides distasonis	6518	NT	>256	>128	128	256	256	64	<0.12
Fusobacterium nucleatum	6324	NT	128	64	64	16	256	4	<0.12
Propionibacterium acnes	6564	NT	256	32	8	4	64	2	<0.12
Clostridium perfringens	6509	>128	>256	>128	128	128	>256	256	<0.12
Peptococcus variabilis	6320	NT	>256	64	256	128	256	128	1
Streptococcus pyogenes‡	152	256	>512	NT	>160	80	NT	NT	NT
*Escherichia coli**	9379	>256	>512	NT	>160	>160	NT	NT	NT

Abbreviations: NT, not tested; AZ, aztreonam; Cl, clindamycin; control is Moxalactam, MIC‡= 0.5 μg/mL; MIC*=0.06 μg/mL; † = tested against *B. thetaiotaomicron* UC 9014 (clindamycin = 2 μg/mL); †† = *vs B. fragilis* UC9370 (clindamycin = 1 μg/mL). MICs were determined using a standardized microbroth dilution method.

TABLE II. ZONES OF INHIBITION (Diameter mm, 1 mg/mL, 80 μL/12.7 mm disk).

COMPOUND	*Bf*	*Cp*	*Sa* 6685*	*Sa* 3665	*Ma*	*Se**
2a	154	105RC	77	44	29	67
4e	29	0	0	0	0	0
4f	31	0	8	18	0	Tr
4g	30	0	12	19	19	7
4h	21	0	0	0	21	0
4i	0	Tr	0	16	Tr	0
4j	0	0	0	0	15	0
4m	52	40RC	27	27	22	19
4n	37	36	39	30	22	32
4o	0	20	20H	26H	0	14H
4p	17	0	Tr	0	0	0
4r	88	78	39	36	21	31
5b	52	36H	0	0	0	0
5c	78	33H	0	0	0	0
5d	165	>100RC	9	36	16	23
5e	>100	63	21H	28	19H	20H
5f	24	33H	0	18H	0	0
5g	140	68RC	24H	31H	0	8
5i	76	45RC	Tr	19	0	Tr
5j	36	19	0	0	0	0
5k	65	35RC	0	0	0	0
5l	130	79	38H	48	18	31
6b	22	22H	0	0	0	0
6c	31	29RC	0	0	0	0
6d	25	30	0	0	0	0
6g	0	23RC	0	0	15H	0
6h	27	28RC	0	0	Tr	0
7a	20	0	0	0	15H	0
7b	36	0	0	0	0	0
7c	41	33	Tr	0	0	14H
Clindamycin*	30	24	0	0	Tr	22

Abbreviations: ***Bf***, *Bacteroides fragilis* UC6513; ***Cp***, *Clostridium perfringens* UC6509; ***Sa* 6685**, *Staphylococcus aureus* UC6685[12]; ***Sa* 3665**, *S. aureus* UC3665; ***Ma***, *Mycobacterium avium* UC159; ***Se***, *Staphylococcus epidermidis* UC719; RC, resistant colonies present; H, hazy zone; Tr = trace; * = 6.5 mm disk used.

diol **8**, were antibacterially-inactive. The (*RR,SS*) dihydro compounds **5** were much more active than the corresponding Z-olefinic analogs **4**, in particular for the anaerobes (note the immensity of the zones for **5d, 5e, 5g, 5l**). While the (*RS,SR*) dihydro compounds **7** were more active than their E-olefinic progenitors **6**, the activity level was far less than the diastereomeric **5** series. The acetamidoisopropyl derivative **5i** was less active than the acetoxyisopropyl bioisostere **5d**. E Alcohol **6h** demonstrated weak antibacterial activity. Compounds **5d** and **5l** were the most potent analogs in this study, albeit both were somewhat less active than the previously-described unsubstituted isopropyl parent **2a**[1] {X=CH_2, R=$(CH_2)_3CH_3$}. These lipophilic compounds generally demonstrated atypically-large zones of inhibition.[1]

In summary, a variety of C-3 functionalized derivatives are accessible through modification of 3-(hydroxyisopropylidenyl)azetidin-2-ones. While N-sulfonated compounds are inactive, the N-acyl azetidinones have potent *in vitro* activity *vs* anaerobes (particularly *Bacteroides* species) and Gram-positive aerobes, yet do not contain acidic or charged moieties appended to N-1.[13]

Acknowledgement: The authors are grateful to Ms. Betty R. Yagi for *in vitro* technical assistance, and Dr. Richard C. Thomas for helpful discussion.

References and Notes

1. Brickner, S. J.; Gaikema, J. J.; Zurenko, G. E.; Greenfield, L. J.; Manninen, P. R.; Ulanowicz, D. A. *J. Antibiotics* **1992**, *45*, 213.
2. Virudachalam, R.; Rao, V. S. R. *Biomolecular Structure, Conformation, Function and Evolution*; Vol. 2, Srinivasan, Ed.; Pergamon Press: Oxford, 1980, 525.
3. Firestone, R. A.; Barker, P. L.; Pisano, J. M.; Ashe, B. M.; Dahlgren, *Tetrahedron* **1990**, *46*, 2255.
4. Waxman, D. J.; Strominger, J. L. *J. Biol. Chem.* **1980**, *255*, 3964.
5. Brickner, S. J.; Gaikema, J. J.; Torrado, J. T.; Greenfield, L. J.; Ulanowicz, D. A. *Tetrahedron Lett.* **1988**, *29*, 5601.
6. Imada, A.; Kitano, K.; Kintaka, K.; Muroi, M.; Asai, M. *Nature* **1981**, *289*, 590. Sykes, R. B.; Cimarusti, C. M.; Bonner, D. P.; Bush, K.; Floyd, D. M.; Georgopapadakou, N. H.; Koster, W. H.; Liu, W. C.; Parker, W. L.; Principe, P. A.; Rathnum, M. L.; Slusarchyk, W. A.; Trejo, W. H.; Wells, J. S. *Nature* **1981**, *291*, 489.
7. Nishikawa, J.; Tori, K. *J. Antibiotics* **1981**, *34*, 1641, 1645.
8. Shoji, J.; Hinoo, H.; Sakazaki, R.; Tsuji, N.; Nagashima, K.; Matsumoto, K.; Takahashi, Y.; Kozuki, S.; Hattori, T.; Kondo, E.; Tanaka, K. *J. Antibiotics* **1982**, *35*, 15.
9. For a related γ-oxo allylic alkylation see: Keinan, E.; Sahai, M.; Kirson, I. *J. Org. Chem.* **1983**, *48*, 2550.
10. Facile isomerization of π-allyl palladium intermediates *via* a σ-bonded palladium complex has been reported. *Cf*: Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4730.
11. Partial disclosure of this data has been previously presented: Brickner, S. J.; Gaikema, J. J.; Engelhardt, L. J.; Mydlow, P. K.; Zins, B. J.; Torrado, J. T. Abstract No. 1217, 27th Intersci. Conf. on Antimicrob. Agents Chemother., p. 309, New York, October 4-7, 1987. Brickner, S. J.; Gaikema, J. J.; Greenfield, L. J.; Mydlow, P. K.; Ulanowicz, D. A.; Manninen, P. R. Abstract No. P 11, p. 57, 4th Intern. Symp.: Recent Advances in the Chemistry of β-Lactam Antibiotics, Cambridge UK, July 3-6, 1988.
12. *Staphylococcus aureus* UC®6685 is resistant to multiple antibiotics, including: penicillin, methicillin, cephalothin, tetracycline, clindamycin, novobiocin, kanamycin, and erythromycin.
13. Studies concerning the mechanism of action and β-lactamase stabilities of these unusual β-lactams will be reported elsewhere.