Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1373–1375

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Azalide 3,6-Ketals: Antibacterial Activity and Structure–Activity Relationships of Aryl and Hetero Aryl Substituted Analogues

Subas M. Sakya,* Peter Bertinato, Bryan Pratt, Melani Suarez-Contreras, Kristin M. Lundy, Martha L. Minich, Hengmiao Cheng, Carl B. Ziegler, Barbara J. Kamicker, Shigeru F. Hayashi, Sheryl L. Santoro, David M. George and Camilla D. Bertsche

Veterinary Medicine Research and Development, Pfizer Inc, Eastern Point Road, Box 4063, Groton, CT 06340, USA

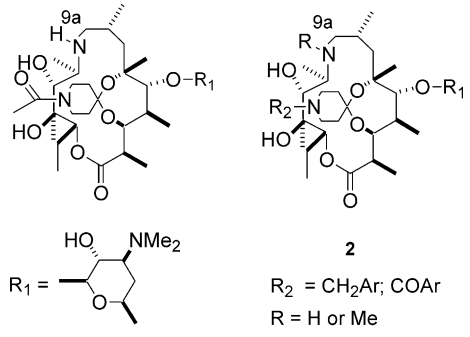
Received 17 September 2002; accepted 16 December 2002

Abstract—Aryl and hetero aryl substituted 3,6-ketals of 15-membered azalide analogues were synthesized and were found to have potent in vitro antibacterial activity against veterinary pathogens, including *Staphylococcus aureus* and *Pasteurella multocida*.

Mastitis is an inflammation of the mammary gland, usually caused by a variety of bacteria. One of the most prevalent pathogens for non-lactating cow (dry cow) mastitis is *Staphylococcus aureus*. Mastitis is the most important disease in the worldwide dairy industry and causes considerable economic loss. The US National Mastitis Council estimates that annual losses to the dairy industry amount to US$1.8–2 billion, or US$185–200 per cow. Yearly, between 30 and 50% of all dairy cattle in the US are affected by mastitis.[1,2]

Antibiotic therapy is widely used to eliminate mastitis-causing infections and return udders to a healthy condition. These antibacterial agents include penicillins, tetracyclines, macrolides and a number of cephalosporins. Intramammary infusion (IMI) is commonly used to deliver the antibiotics. However, none of the treatments is significantly effective against mastitis caused by *S. aureus*. Here, we report our attempts to discover antibacterial agents with improved activity against *S. aureus* when administered by subcutaneous injection.

In the search for active compounds against bovine respiratory disease (BRD), Lundy et al. discovered that 3,6-ketals of the 9a-azalides showed excellent in vitro activity against the Gram-negative pathogens, *Pasteurella multocida* and *Mannheimia* (*Pasteurella*) *haemolytica*, responsible for BRD.[3] These analogues, exemplified by **1**, also showed potent in vivo activity against those pathogens.[4] We initiated synthetic efforts to identify compounds with improved activity against Gram-positive mastitis pathogens using the 3,6-ketal azalide as a template. Recently we reported the SAR of the benzyl and aryl amide modifications on the 3,6-ketal nitrogen (Fig. 1) in which we showed improved activity by incorporation of heteroaryl moieties.[4] In this paper, we will describe the results of the modification of the piperidine ketal nitrogen with aryl and heteroaryl derivatives.



CP-456280 (**1**)

Figure 1. Lead 3,6-piperidine ketal azalides.

*Corresponding author. Fax: +1-860-715-7312; e-mail: sakyas@groton.pfizer.com

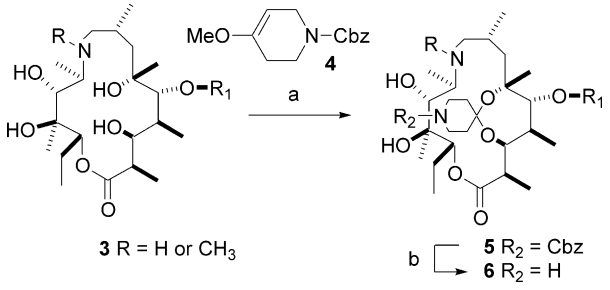
0960-894X/03/$ - see front matter
doi:10.1016/S0960-894X(03)00100-8

Compound **6** was used as precursor material for synthesis of the 3,6-ketal analogues. The synthesis of **6** was carried out using established procedures according to Scheme 1.[4]

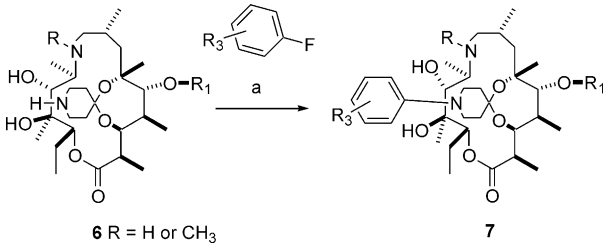
The syntheses of aryl piperidine ketal derivatives were accomplished by heating 3,6-ketal **6** with aryl fluorides in the presence of base as shown in Scheme 2.

Scheme 3 shows the preparation of the 2-pyridyl, 2-pyrimidine, and 2-triazine derivatives. Generally moderate to good yields of the products were obtained. Similar reaction conditions were used for the preparation of other heterocyles (**8t** and **8v**–**8y**). The pyrimidine groups were further functionalized to make several ester and amide derivatives.

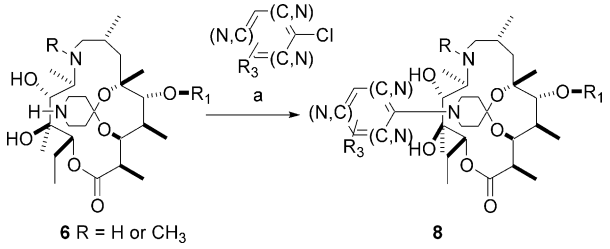
While broad spectrum agents are desirable, activity against the Gram-positive bacteria *S. aureus* is essential. Among the substituted aryl analogues, 2-fluoro or 3-CF_3-4-benzyloxycarbonyl substituted derivatives (**7a** and **7c**) with 9a *N*-H showed potent activity against *S. aureus* (MIC: 0.39–0.78 μg/mL) but had less activity against the Gram-negative *Escherichia coli*. The corresponding 3-CF_3-4-benzyloxycarbonyl aryl derivative (**7b**) with 9a *N*-Me showed improved activity against *E. coli*. The nitrophenyl derivatives (**7d** and **7e**) showed only moderate activity against all three pathogens. Other functional groups containing aryl analogues did not improve upon the activity against *S. aureus*.



Scheme 1. (a) pTsOH, **4**, CH_2Cl_2, 60%; (b) H_2, Pd/C, 99%.



Scheme 2. (a) $CsCO_3$, aryl fluoride, base, 80–95 °C, 60–85%.



Scheme 3. (a) Et_3N, 2-chloro pyridine, 2-chloro pyrimidine, or 2-chloro triazine, CH_3CN or *i*PrOH 80–95 °C, 60–85%.

Among the pyridine analogues, nitro- and cyano-substituted analogues (**8a**–**8c**) showed good activity against *S. aureus* and *P. multocida*, and reduced activity against *E. coli*. Adding a more polar functionality to the molecule (**8d**) improved the activity against *E. coli*. The hydroxy methyl substituted analogue (**8e**) had the best activity against all three pathogens. Most analogues had reasonable activity against all the pathogens with the exception of **8k** (Table 1).

The pyrimidine analogues had the weakest activity against *E. coli* and *P. multocida* while having good activity against *S. aureus*. The more lipophilic **8l** and **8m** had the best activity against *S. aureus*. Several of the pyrimidine analogues (**8n**, **8o**, **8r**, and **8s**) had moderate activity against all three pathogens. Further modifications of the pyrimidine ring did not improve activity.

The heterocyclic analogues **8s**–**8x** did not show any enhanced activity over the other analogues. Overall, very slight differences in the activity profile of the various aryl and heteroaryl derivatives of the 3,6-piperidine ketals were seen. A majority of the aryl and heteroaryl analogues showed improved activity against *S. aureus* over the unsubstituted 3,6-ketal **6** and reduced activity against *E. coli*. With the exception of the rather lipophilic pyrimidine analogues, most analogues were active against *P. multocida*. Several of the active aryl, pyridine and pyrimidine analogues (**7a**, **7b**, **7d**, **7e**, **8a**, **8f**, **8h**, **8l**, **8m**, **8o**, **8r**, and **8u**) were tested in a *S. aureus* murine intraperitoneal infection model and showed poor in vivo activity (>20 mg/kg ED_{50}). Previous studies[5] had shown that polarity was essential for improved in vivo activity for macrolides. Therefore, the lack of in vivo activity may be due to the significantly increased lipophilicity of these new analogues.

Aryl and heteroaryl linked piperidine 3,6-ketal azalides with potent in vitro activity against both Gram-positive and Gram-negative bacteria were discovered. There was no significant difference in the in vitro activity against *S. aureus* between 9a *N*-H and *N*-Me analogues. Unlike the previously reported compounds[4] these lipophilic derivatives had minimal or no activity in a *S. aureus* in vivo murine model.

In Vitro Assays

The microdilution method based on the NCCLS guidelines[6] has been reported previously.[4] Minimum inhibitory concentration (MIC) was determined at the lowest drug concentration that completely inhibits bacterial growth. The following three strains were used for the MIC determinations: 01A0758 (*S. aureus*), 51A0150 (*E. coli*), and 59A0067 (*P. multocida*).

Table 1. Biological data for aryl and hetero aryl piperidine amine-3,6-ketals

Compd	Ar	MIC (μg/mL)				
		R (9a)	*S. aureus*	*E. coli*	*P. multocida*	Clog*P*
6	N/A	Me	6.25	0.39	<0.05	1.454
7a	2-Fluoro-4-benzyloxycarbonyl phenyl	H	0.39	>50	3.13	5.529
7b	3-CF_3-4-Benzyloxycarbonyl phenyl	Me	0.78	3.13	0.39	7.61
7c	3-CF_3-4-Benzyloxycarbonyl phenyl	H	0.78	>50	6.25	6.757
7d	2-Nitro phenyl	Me	1.56	3.13	0.39	4.698
7e	4-Nitro phenyl	Me	1.56	6.25	0.39	4.698
7f	2-Fluoro-4-pyridyl methyl amido phenyl	Me	3.13	1.56	0.2	3.754
7g	4-Cyano phenyl	Me	3.13	25	0.39	4.419
7h	3-CF_3-4-COOH-phenyl	Me	25	12.5	3.13	2.554
7i	4-Benzyloxycarbonyl phenyl	Me	3.13	3.13	0.39	6.638
7j	4-(4-F-Phenylsulfonyl) phenyl	Me	3.13	>50	1.56	5.573
7k	4-Benzoylamino phenyl	Me	12.5	50	1.56	5.097
7l	4-Amino phenyl	Me	6.35	12.5	1.56	3.362
8a	3-Nitro-2-pyridyl	H	0.78	6.25	0.20	2.695
8b	3-Cyano-6-Me-2-pyridyl	H	0.78	6.25	0.78	2.898
8c	4-Methyl-5-nitro-2- pyridyl	H	0.78	6.25	1.56	3.114
8d	5-Nitro-6-amino-2-pyridyl	H	1.56	3.13	0.1	2.974
8e	4-Hydroxymethyl-6-chloro-2-pyridyl	H	1.56	0.78	0.2	2.49
8f	5-Cyano-2-pyridyl	H	1.56	6.25	0.20	2.399
8g	5-Amido-2-pyridyl	H	1.56	6.25	0.20	1.929
8h	5-Nitro-2-pyridyl	H	1.56	3.13	0.39	2.695
8i	5-CF_3-2-pyridyl	H	1.56	12.5	0.78	3.805
8j	5-Nitro-2-pyridyl	Me	3.13	6.25	0.39	3.548
8k	5-Ethoxycarbonyl 2-pyridyl	H	6.25	50	3.13	3.426
8l	4-CF_3-5-Benyloxycarbonyl-2-pyrimidine	H	0.78	>50	>50	4.675
8m	4-CF_3-5-Benyloxycarbonyl-2-pyrimidine	Me	1.17	>50	>50	5.528
8n	2-Pyrimidine	Me	1.56	1.56	0.10	2.849
8o	4-CF_3-2-Pyrimidine	H	1.56	3.13	0.78	2.923
8p	5-(Pyrid-4-ylmethyl amido)-2-pyrimidine	Me	3.52	>50	>50	2.408
8q	4-CF_3-2-Pyrimidine	Me	6.25	12.5	1.56	3.776
8r	2,4-Methoxy-6-pyrimidine	H	1.56	3.13	0.2	3.72
8s	2,4-Methoxy-6-pyrimidine	Me	1.56	6.25	0.39	4.576
8t	6-Chloro-2-pyrazine	Me	1.56	6.25	0.39	3.537
8u	4-Chloro-6-phenyl-2-1,3,5-triazine	Me	1.56	>50	>50	4.63
8v	2-Benzimidazole	Me	3.13	1.56	0.1	4.561
8w	2-Thiazole	Me	3.13	3.13	0.39	3.519
8x	2-Benzoxazole	Me	3.13	6.25	0.78	4.411
8y	6-Methyl-3-pyridazine	Me	6.25	3.13	0.20	3.183

Murine Models

A *S. aureus* intraperitoneal infection model was described in a previous report.[4] The number of surviving mice was counted after 4 days, and the effective dose for 50% survival (ED_{50}) was calculated using a regression equation.

Acknowledgements

We are grateful to Dr. Robert Rafka's group for providing us multi-gram quantities of **6** and Dr. John Dirlam for many helpful discussions.

References and Notes

1. (a) Bramley, A. J.; Cullor, J. S.; Erskine, R. J.; Fox, L. K.; Harmon, R. J.; Hogan, J. S.; Nickerson, S. C.; Oliver, S. P.; Smith, L. K.; Sordillo, L. M. In *Current Concepts of Bovine Mastitis;* 4th ed. National Mastitis Council, 1996; p 11. (b) Barkema, H. W.; Schukken, Y. H.; Lam, T. J. M.; Beiboer, M. L.; Wilmink, H.; Benedictus, G.; Brand, A. *J. Dairy Sci.* **1998**, *81*, 411.

2. (a) Hogan, J. S.; Smith, K. L.; Hoblet, K. H.; Schoenberger, P. S.; Todhunter, D. A.; Hueston, W. D.; Pritchard, D. E.; Bowman, G. L.; Heider, L. E.; Brockett, B. L.; Conrad, H. R. *J. Dairy Sci.* **1989**, *72*, 1547. (b) Clements, M. In *Animal Pharm Reports, Bovine Mastitis: Products and Markets*; PJB, Richmond, UK: June 30, 1998 p 9.

3. (a) Minich, M. L.; Lundy, K. M. *Abstract of Papers*, Structural Elucidation of Descladinose Azithromycin-3,6-(Azacyclohexyl) Ketal by One- and Two-D NMR Spectroscopy, 218th National Meeting of the American Chemical Society, New Orleans, LA, Aug 22–26, 1999. (b) Minich, M. L.; Lundy, K. M.; Rafka, R. J.; Morton, B. J. *Abstract of Papers*, Azalide 3,6-Ketals: Discovery of a Novel Class of Azalide Antibiotics, 218th National Meeting of the American Chemical Society, New Orleans, LA, Aug 22–26, 1999.

4. Cheng, H.; Dirlam, J. P.; Ziegler, C. B.; Lundy, K. M.; Hayashi, S. F.; Kamicker, B. J.; Dutra, J. K.; Daniel, K. L.; Santoro, S. L.; George, D. M.; Bertsche, C. D.; Sakya, S. M.; Suarez-Contreras, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2431.

5. McFarland, J. W.; Berger, C. M.; Froshauer, S. A.; Hayashi, A. F.; Hecker, S. J.; Jaynes, B. H.; Jefson, M. R.; Kamicker, B. J.; Lipinski, C. A.; Lundy, K. M.; Reese, C. P.; Vu, C. B. *J. Med. Chem.* **1997**, *40*, 1340.

6. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard, M31-A, Vol. 19, No. 11, June, 1999.