Bioorganic & Medicinal Chemistry Letters 20 (2010) 4635–4638

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Propenylamide and propenylsulfonamide cephalosporins as a novel class of anti-MRSA β-lactams

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ARTICLE INFO

Article history:
Received 3 May 2010
Revised 27 May 2010
Accepted 29 May 2010
Available online 8 June 2010

Keywords:
Cephalosporins
MRSA

ABSTRACT

Novel C(3) propenylamide and propenylsulfonamide cephalosporins have been synthesized and tested for their ability to inhibit the penicillin-binding protein 2′ (PBP2′) from *Staphylococcus epidermidis* and the growth of a panel of clinically relevant bacterial species, including methicillin-resistant *Staphylococcus aureus* (MRSA). The most potent compounds inhibited the growth of MRSA strains with minimum inhibitory concentrations (MIC) as low as 1 μg/mL. The structure–activity relationship revealed the potential for further optimization of this new cephalosporin class.

Cephalosporins have a history of success in the treatment of bacterial infections. Their favorable safety profile and generally bactericidal mode of action have made them one of the most frequently used classes of antibacterial drugs.[1] However, the emergence of resistance has created the need for new antibacterial compounds.[2,3] Therefore, an extensive search for cephalosporins with efficacy also against resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA), has been made in recent years.[4] These efforts yielded a number of anti-MRSA cephalosporins, with the most advanced representative, ceftobiprole, recently being approved in first countries.[5,6]

A common feature of most anti-MRSA cephalosporins is the presence of a basic group, or even a permanent positive charge in the C(3) side-chain.[7,8] This positive charge increases the apparent binding affinity of the drug towards PBP2′, the enzyme that is the main cause for the resistance of staphylococci towards older generations of penicillinase-stable β-lactams.[9,10] A special subgroup among these charged cephalosporins comprises derivatives with a quaternary ammonium moiety that is linked to the cephalosporin core via a propenyl group.[11–14] The favorable activity of these compounds against MRSA can be attributed to the leaving group properties of the ammonium moiety upon reaction with penicillin-binding proteins (Fig. 1). However, the zwitterionic nature of these drugs can lead to an unfavorable physico-chemical profile that poses challenges for further development.

Here, we report for the first time non-basic, uncharged C(3) propenylamide and propenylsulfonamide cephalosporins with potent anti-MRSA activity that is not attributable to a leaving group in these novel side-chains.

Cephalosporin building block **6** served as key intermediate for all propenylamide and propenylsulfonamide cephalosporins in this study. Orthogonally protected **6** was prepared from 3-hydroxy cephalosporin **4** by formation of the corresponding triflate **5** and subsequent palladium acetate catalyzed Stille coupling with the *E*-vinylstannane **3** to introduce the *N*-Boc-protected aminopropenyl side-chain (Scheme 1).[15] Vinylstannane **3** was obtained stereoselectively by hydrostannylation of the corresponding propargylamine **2**.[16]

Treatment of **6** with trifluoroacetic acid allowed simultaneous deprotection of the amino and carboxylic acid moieties, thus providing cephalosporin **7**. Different approaches were evaluated for the acylation of the terminal amino group of **7**. Reaction of **7** with aromatic acylchlorides proceeded rapidly, but with poor chemoselectivity for the amino versus the carboxylic acid group. Coupling by activating the acid as a mixed-anhydride with methyl- or *i*-butyl-chloroformate worked well for aliphatic acids, but led to mixtures of the desired product and the corresponding carbamate when aromatic acids were applied. However, employing CDI or TBTU as coupling reagent gave good yields for both aliphatic and aromatic acids.

The preparation of sulfonamides was achieved by treatment of **7** with sulfonyl chlorides. The choice of base greatly affected the outcome of these reactions: DIPEA, for example, led to complex product mixtures, whereas the use of BSA allowed the reproducible preparation of the desired products in good yield.

To obtain cephalosporins with variations in the 7-aminoacyl moiety, the phenylacetyl group of **6** was removed by the

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doi:10.1016/j.bmcl.2010.05.110

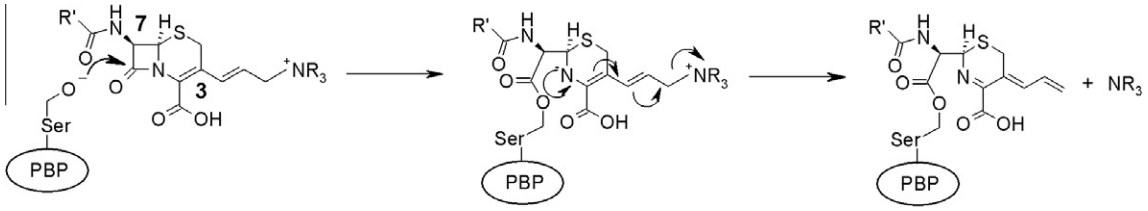
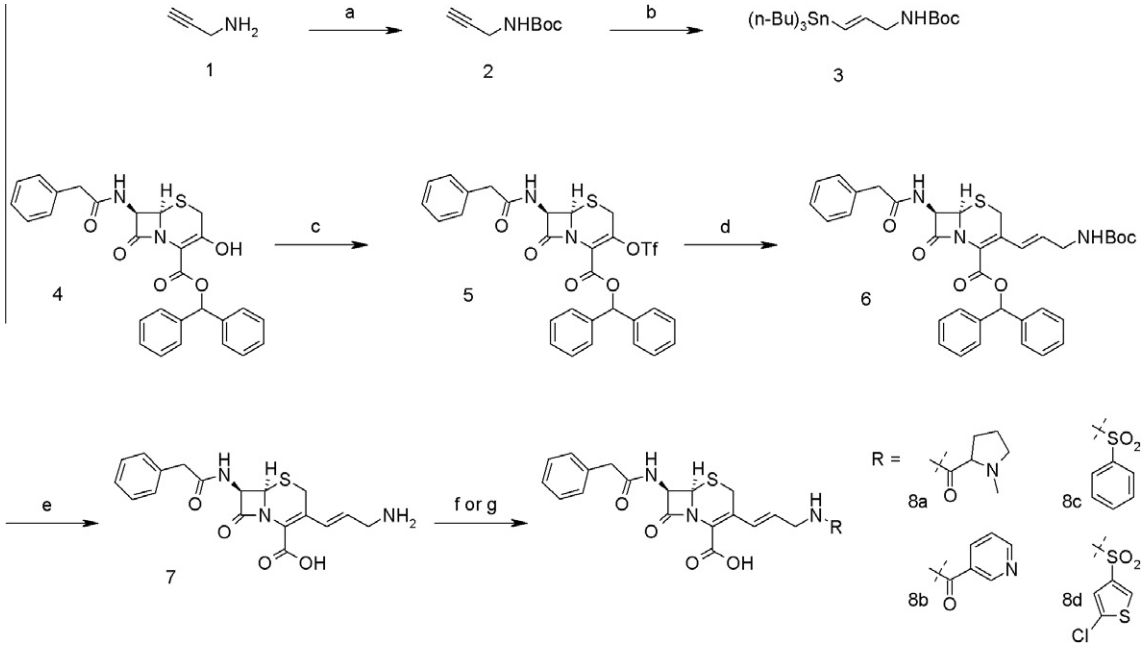


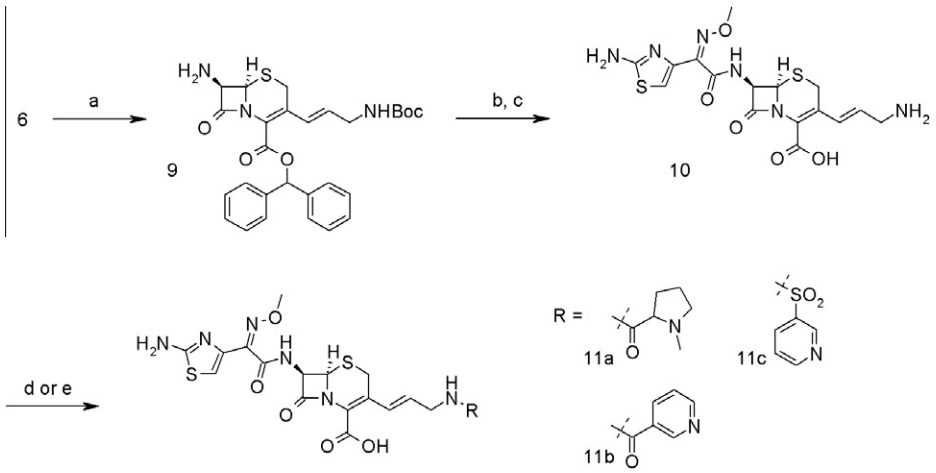
Figure 1. Reaction of propenylammonium cephalosporins with penicillin-binding proteins.



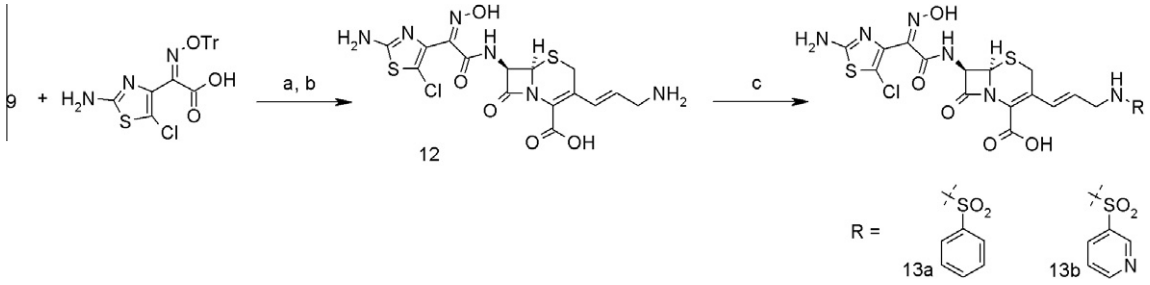
Scheme 1. Reagents and conditions: (a) Boc_2O, DIPEA, CH_2Cl_2, rt; (b) *n*-Bu_3SnH, cat. AIBN, benzene, 80 °C; (c) Tf_2O, DIPEA, CH_2Cl_2, −78 °C; (d) **3**, cat. $Pd(OAc)_2$, NMP, rt; (e) TFA, CH_2Cl_2, rt; (f) RCO_2H, CDI, NMP, rt; (g) RSO_2Cl, BSA, CH_2Cl_2, reflux.

standard imino-chloride method.[17] Careful evaluation of the subsequent acylation conditions led to the selection of TBTU or PyBOP as preferred coupling reagents that precluded isomerization of the cephalosporin Δ_2–Δ_3 double bond. Aminothiazolyl-oximino acetyl and aromatic thioacetyl side-chains were introduced by this method. Subsequent deprotection and derivatization of the propenylamino group were carried out as described above for the 7-phenylacetamide cephalosporins (Schemes 2–4).

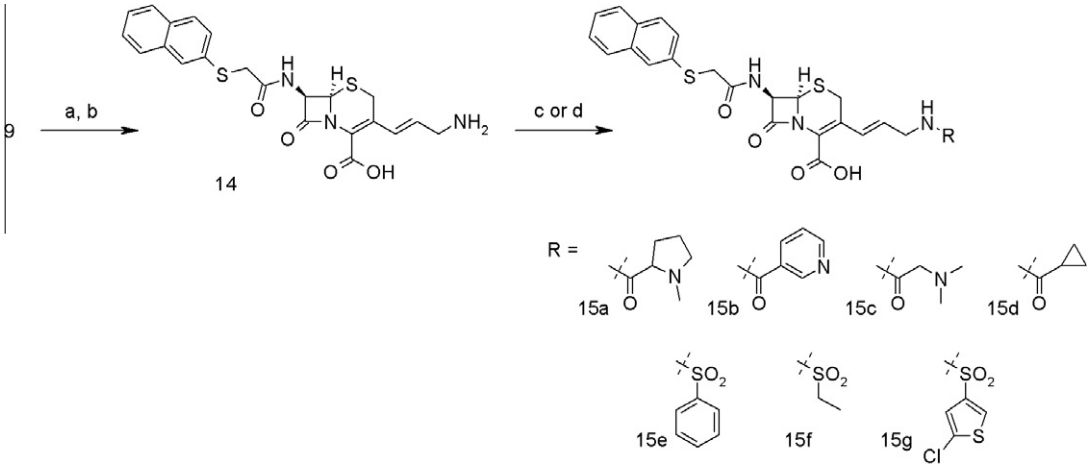
All new propenylamide and propenylsulfonamide cephalosporins were tested for their ability to inhibit purified recombinant PBP2′ from *Staphylococcus epidermidis* and for their activity against a panel of bacterial strains, comprised of *S. aureus*, *Streptococcus pneumoniae* and *Escherichia coli*.[18,19]



Scheme 2. Reagents and conditions: (a) (1) PCl_5/Py, CH_2Cl_2, −10 °C; (2) MeOH, −20 to 10 °C; (b) (2-amino-thiazol-4-yl)-methoxyimino-thioacetic acid *S*-benzothiazol-2-yl ester, NMP, rt; (c) TFA, CH_2Cl_2, rt; (d) RCO_2H, CDI, NMP, rt; (e) RSO_2Cl, BSA, CH_2Cl_2, reflux.



Scheme 3. Reagents and conditions: (a) TBTU, DIPEA, NMP, rt; (b) TFA, CH_2Cl_2, rt; (c) RSO_2Cl, BSA, CH_2Cl_2, reflux.



Scheme 4. Reagents and conditions: (a) (2-Naphthylthio)acetic acid, TBTU, DIPEA, NMP, rt; (b) TFA, CH_2Cl_2; (c) RCO_2H, CDI, NMP, rt; (d) RSO_2Cl, BSA, CH_2Cl_2, reflux.

Table 1 shows that cephalosporins with a simple C(7) phenylacetamide side-chain exhibited good antibacterial activity against sensitive *S. aureus* and *S. pneumoniae*, but that there was generally no significant activity against MRSA (*S. aureus* COL BLA$^-$) and *E. coli*. Replacement of the C(7) phenylacetamide side-chain by an aminothiazolyl-methoxyimino acetamide group gave some activity against *E. coli* (compounds **11a**–**11c** in Table 2), but resulted in poor inhibition of PBP2′ and decreased activity against sensitive *S. aureus* strains. However, this is a known effect of the methyl-substituted oximino group.[21,22] Therefore, the correspond-

Table 1
MIC values of C(7) phenylacetamide cephalosporins

Compound	MIC (μg/mL)					
	S. aureus				*S. pneumoniae*	*E. coli*
	ATCC 25923	887[a]	COL BLA$^-$[20]	COL BLA$^+$[a]	R6	UB1005
8a	0.25	8	>32	>32	⩽0.06	>32
8b	⩽0.06	n.d.[b]	>32	>32	⩽0.06	>32
8c	0.5	16	>32	>32	0.13	>32
8d	0.13	2	32	>32	⩽0.06	>32
Amoxicillin	0.25	32	32	>32	⩽0.06	8
Cephalothin	0.25	0.5	>32	>32	0.125	8

[a] β-Lactamase producer.
[b] Not done.

Table 2
IC_{50} and MIC values of C(7) aminothiazolyl-oximino acetamide cephalosporins

Compound	IC_{50} (μM)	MIC (μg/mL)					
	PBP2′	*S. aureus*				*S. pneumoniae*	*E. coli*
		ATCC 25923	887	COL BLA$^-$	COL BLA$^+$	R6	UB1005
11a	>500	2	8	>32	>32	⩽0.06	1
11b	>500	4	8	>32	>32	⩽0.06	0.25
11c	235 ± 110	1	2	>32	>32	⩽0.06	1
13a	6 ± 0.4	0.5	1	8	16	0.25	>32
13b	12 ± 2	2	4	16	32	⩽0.06	16

Table 3
IC_{50} and MIC values of C(7) thioacetamide cephalosporins

Compound	IC_{50} (μM)	MIC (μg/mL)						
	PBP2′	*S. aureus*					*S. pneumoniae*	*E. coli*
		ATCC 25923	887	COL BLA$^-$	COL BLA$^+$	COL BLA$^+$ (+4 μg/mL sulbactam)	R6	UB1005
15a	129 ± 8	0.13	1	4	>32	1	0.13	>32
15b	31 ± 16	0.13	1	4	>32	1	⩽0.06	>32
15c	50 ± 6	⩽0.06	1	4	32	4	⩽0.06	>32
15d	69 ± 16	⩽0.06	0.5	1	16	n.d.	⩽0.06	>32
15e	20 ± 1.5	⩽0.06	2	1	32	n.d.	⩽0.06	>32
15f	22 ± 8	⩽0.06	2	8	>32	n.d.	⩽0.06	>32
15g	2 ± 0.5	⩽0.06	0.5	1	16	1	⩽0.06	>32

ing chloro-aminothiazolyl-hydroxyimino acetamide derivatives **13a** and **13b** were evaluated, and, indeed, these compounds exhibited significantly increased affinities towards PBP2′, which translated into MICs as low as 8 μg/mL against MRSA. Compounds **13a** and **13b** were apparently penicillinase-stable as the MICs were not significantly affected by the presence of the staphylococcal PC-1 β-lactamase. Further improvement of anti-MRSA activity was achieved by introducing an aromatic thioacetamide side-chain at C(7) (Table 3). The growth of *S. aureus* COL BLA$^-$ was inhibited at concentrations as low as 1 μg/mL with still an excellent coverage of sensitive Gram-positive bacteria. In general, it was interesting to note that a relatively broad structure–activity relationship (SAR) was observed for the propenyl substituent. Aromatic and non-aromatic amides and sulfonamides showed similar antibacterial activity that was apparently independent of the presence of a basic moiety in the side-chain. A disadvantage of the thioacetamide side-chain is that it confers lability towards β-lactamases, as seen by the increased MIC values for PC-1 β-lactamase-producing strain *S. aureus* COL BLA$^+$. Combination with a β-lactamase inhibitor would be a feasible option to counter this problem. Indeed, addition of sulbactam rendered the β-lactamase-producing strain of *S. aureus* sensitive towards the propenylamide and propenylsulfonamide cephalosporins.

It was mentioned above, that the activity of other C(3) propenyl cephalosporins is based on the leaving group properties of the appropriate substituent, as this contributes to the stabilization of the acyl–enzyme complex once it has formed. Amides and sulfonamides are not good leaving groups and it was therefore questionable whether they are released during the reaction with PBPs. To evaluate this, **15b** was treated with purified IMP1 β-lactamase[23] and the resulting mixture was checked for 3-pyridylcarboxamide, which should have been formed if the reaction had occurred.[24] In these experiments, no free 3-pyridylcarboxamide was found despite the successful hydrolysis of the cephalosporin by IMP1 β-lactamase, indicating that the enhanced anti-MRSA activity of the novel propenylamide and, by inference, propenylsulfonamide cephalosporins is not due to leaving group characteristics of the C(3) side-chain substituent.

In summary, we have identified propenylamide and propenylsulfonamide cephalosporins as members of a new subclass of antibacterial β-lactams. Depending on the nature of the C(7) side-chain, there is the potential to optimize either for activity against MRSA or enterobacteriaceae. The broad SAR observed for the propenyl substituent allows optimization of potency as well as of the physico-chemical profile, thus prompting further evaluation to discover the full potential of this novel cephalosporin class.

Acknowledgments

The authors would like to thank Elena I. Mayboroda and Inna Yankovich for their excellent support in the synthesis of the compounds and Laure Thenoz for the determination of the MIC values.

References and notes

1. Mitropoulos, I. F.; Rotschafer, J. C.; Rodvold, K. A. *Diagn. Microbiol. Infect. Dis.* **2007**, *57*, 67S.
2. Appelbaum, P. C. *Clin. Microbiol. Infect.* **2006**, *12*, 3.
3. Barrett, J. F. *Expert Opin. Ther. Targets* **2004**, *8*, 515.
4. Livermore, D. M. *Clin. Microbiol. Infect.* **2006**, *12*, 11.
5. Bush, K.; Heep, M.; Macielag, M. J.; Noel, G. J. *Expert Opin. Invest. Drugs* **2007**, *16*, 419.
6. Page, M. G. P. *Expert Opin. Drug Discov.* **2007**, *2*, 115.
7. Page, M. G. P. *Expert Opin. Invest. Drugs* **2004**, *13*, 973.
8. Glinka, T. W. *Curr. Opin. Invest. Drugs* **2002**, *3*, 206.
9. Fuda, C.; Suvorov, M.; Vakulenko, S. B.; Mobashery, S. *J. Biol. Chem.* **2004**, *279*, 40802.
10. Guignard, B.; Entenza, J. M.; Moreillon, P. *Curr. Opin. Pharmacol.* **2005**, *5*, 479.
11. Kamachi, H.; Oka, M.; Narita, Y.; Rimura, S.; Aburaki, S.; Yamashita, H.; Tomatsu, K.; Okumara, J.; Naito, T. *J. Antibiot.* **1990**, *43*, 533.
12. Toyosawa, T.; Miyazaki, S.; Tsuji, A.; Yamaguchi, K.; Goto, S. *Antimicrob. Agents Chemother.* **1993**, *37*, 60.
13. Lee, J. Y.; Lee, Y. S.; Suk, D. W.; Woo, E.-R.; Chung, B. Y.; Park, H. *J. Antibiot.* **1996**, *49*, 1286.
14. Angehrn, P.; Goetschi, E.; Heinze-Krauss, I.; Richter, H. G. F. WO 9967255, 1999.
15. Baker, S. R.; Roth, G. P.; Sapino, C. *Synth. Commun.* **1990**, *20*, 2185.
16. Kende, A. S.; DeVita, R. J. *Tetrahedron Lett.* **1990**, *31*, 307.
17. Yamanaka, H.; Chiba, T.; Kawabata, K.; Takasugi, H.; Masugi, T.; Takaya, T. *J Antibiot.* **1985**, *38*, 1738.
18. Hebeisen, P.; Heinze-Krauss, I.; Angehrn, P.; Hohl, P.; Page, M. G. P.; Then, R. L. *Antimicrob. Agents Chemother.* **2001**, *45*, 825.
19. National Committee for Clinical Laboratory Standards. In *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 7th ed.; Approved Standard CLSI Publication No. M7-A7; Clinical and Laboratory Standards Institute: Wayne, PA, 2006.
20. Vouillamoz, J.; Entenza, J. M.; Hohl, P.; Moreillon, P. *Antimicrob. Agents Chemother.* **2004**, *48*, 4322.
21. Glinka, T. W.; Cho, A.; Zhang, Z. J.; Ludwikow, M.; Griffith, D.; Huie, K.; Hecker, S. J.; Dudley, M. N.; Lee, V. J.; Chamberland, S. *J. Antibiot.* **2000**, *53*, 1045.
22. Ishikawa, T.; Kamiyama, K.; Matsunaga, N.; Tawada, H.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiot.* **2000**, *53*, 1071.
23. Laraki, N.; Franceschini, N.; Rossolini, G. M.; Santucci, P.; Meunier, C.; de Pauw, E.; Amicosante, G.; Frère, J. M.; Galleni, M. *Antimicrob. Agents Chemother.* **1999**, *43*, 902.
24. Compound **15b** was mixed in water with IMP1 β-lactamase. After 5 min of incubation, the reaction was stopped by addition of acetonitrile and the solution was analyzed by LC–UV–MS. In this analysis, **15b** and 3-pyridylcarboxamide were used as reference.