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Allyl and Propargyl Substituted Penam Sulfones as Versatile Intermediates Toward the Syntheses of New β-Lactamase Inhibitors

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Abstract—Several alkenyl derivatives were prepared using allyl penam sulfone as the key intermediate. Isomers of these derivatives having β configuration at C-6 showed potent acitivity against CcrA enzyme. A new method was developed to prepare propargyl penam sulfone. The majority of the triazoles prepared by this route exhibited good activity against all three representative enzymes used for the inhibition assay.

Resistance to antibiotics is a major concern in the infectious disease area.[1] Today, bacterial resistance has developed against vancomycin, which is considered an antibiotic of last resort.[2] β-Lactamases are the primary cause for resistance against most widely used β-lactam antibiotics.[3] These enzymes efficiently hydrolyze the amide bond of the β-lactam ring to give products that are devoid of antibacterial activity. Therefore, the administration of a β-lactamase inhibitor along with an antibiotic represents an important strategy for combating the resistance against these drugs.[4]

Sulbactam (**1**)[5] and tazobactam (**2**)[6] are two currently marketed β-lactamase inhibitors, which are used with an antibiotic in this context (Fig. 1). These inhibitors have potent activity against class A enzymes, but have little or no activity against class B and C enzymes.[7] Therefore, an inhibitor with the broad-spectrum activity is important to fight effectively against emerging resistant pathogenic bacteria. It is known that the reactions of the above inhibitors with β-lactamases involve branched pathways with many putative intermediates, which have been identified.[8] Also, molecular modeling studies of the above inhibitors with several β-lactamases revealed that there is ample space in the active site to accommodate sizable groups at the C-6 position of these molecules. Therefore, we decided to introduce groups at C-6, that might not only increase the binding but also act as reactive moieties toward amino acid residues in the active site. The presumption is that after initial acylation of the enzyme, the resulting acyl–enzyme intermediate can further react with other catalytic site residues leading to an enzyme-bound species.[9] This would result in complete inactivation of the enzyme. In the past few years, several groups[10] have used a similar strategy in which substituents with such implications were introduced at various positions of sulbactam to enhance the broad-spectrum activity. Herein, we report some of our efforts to find an inhibitor that is effective for the most prevalent classes of enzymes.

We envisioned that the penam sulfones containing allyl and propargyl groups at the 6-position would be ideal intermediates that can be easily converted into various targets by functional group manipulations. Allyl substituted penam sulfones and their derivatives were prepared according to Schemes 1–3.

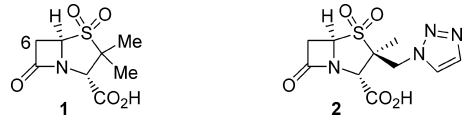

Figure 1. Penam sulfones currently marketed as β-lactamase inhibitors.

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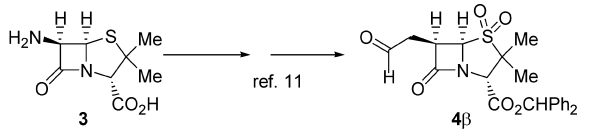
Preparation of compound **4β** has been reported previously, starting with commercially available (+)-6-aminopenicillanic acid (Scheme 1).[11] The corresponding α-isomer **4α** was prepared according to Scheme 2. Dibromide **5** was converted into monobromide **6** in 86% yield using 1 equiv of MeMgBr followed by the aqueous quench. Radical-mediated allyl addition with allyltributyltin gave compound **7** as the sole diastereoisomer in 79% yield.[12] Under these conditions, the tin reagent approaches from the less-hindered α-face to provide the observed stereochemical outcome. Ozonolysis of the allylic compound **7** at −78 °C led to the desired aldehyde **4α** in 69% yield.[13]

Aldehydes **4α** and **4β** were treated with various Wittig reagents to provide the corresponding α,β-unsaturated carbonyl compounds and nitriles **8**–**12** in 60–78% yields (Scheme 3). With the exception of the nitrile, all cases gave (*E*)-isomers as expected. Wittig reaction with nitrile ylide gave a 2:1 mixture of (*E*)- and (*Z*)-isomers, respectively. Conversion of the diphenylmethyl esters to the corresponding acids was achieved by using TFA in trifluoroethanol.

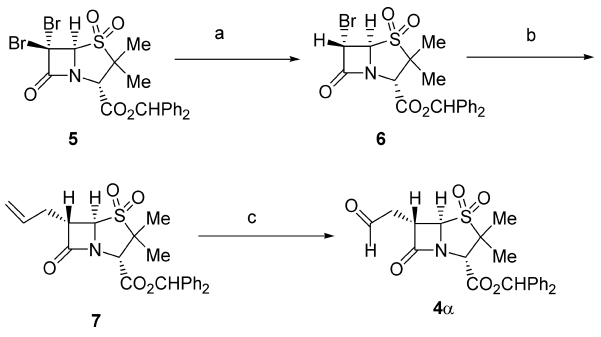
Propargyl substituted penam sulfones and their derivatives were prepared according to Scheme 4. Dibromide **5** was treated with EtMgBr at −78 °C, followed by propargyl aldehyde to give **18** as a 1:1 mixture of diastereoisomers in 61% yield. Debromination with Bu_3SnH/AIBN in toluene at 80 °C provided the desired 6β-isomer **19** in 71% yield. This propargyl derivative **19** underwent smooth dipolar cycloaddition reactions with a series of substituted azides to give the corresponding triazole **20**, as the major regioisomer, in 40–50% yields.[14] Deoxygenation of **20** was effected by forming the thiocarbonate with phenylchlorothiono formate followed by reduction with Bu_3SnH to give the corresponding deoxygenated compound in 18% yield as a single diastereoisomer.[15] Deprotection of the benzhydryl ester by catalytic hydrogenolysis (H_2, Pd/C) gave the target compound **21**. Overall yield of the final products by this method was relatively low mainly due to a poor yield in the deoxygenation step.

To overcome the above deficiency, an alternative synthetic route was devised (Scheme 4). First, dibromide **5** was converted to the corresponding monobromide **6** with EtMgBr at −78 °C as shown in Scheme 2. Initial attempts to obtain compound **23** directly by employing propargyltributyltin under radical conditions provided only the isomeric allenyl product. This observation suggested that if the allenyltributyltin[16] reagent is used under similar conditions, the desired propargyl group could be introduced at the 6-position of the β-lactam ring. Indeed, treatment of monobromide **6** with allenyltributyltin **22** in the presence of AIBN produced the propargyl substituted compound **23**[17] in 50% yield. No allenyl substituted product was detected in the crude reaction mixture. The product crossover in the above reactions is consistent with an S_H' process, which prevails over the direct homolytic substitution (S_H) mechanism.[18] Compound **23** also reacted efficiently with various azides to give the corresponding cycloadducts in 45–70% yields. This method was one step shorter and provided better overall yields compared to the previous method.

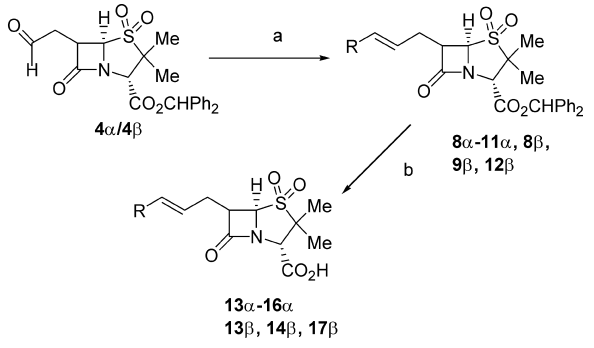
These compounds[19] were evaluated for their inhibitory activity against representative enzymes from three different enzyme classes.[7] While TEM-1 and AmpC are serine-based enzymes, CcrA is a metalloenzyme in which zinc metal plays a key role in initiating catalytic activity. Mechanistically, CcrA follows a unique inhibition pathway as compared to the serine-based enzymes.[20] In addition, metalloenzymes are currently among the quickly spreading β-lactamases especially in Japan and Europe. Our goal was to find an inhibitor that is effective for all three enzymes. Inhibitory activities of these compounds were compared with reference compounds, sulbactam and tazobactam.[21] Overall, the alkenyl series did not show any significant improvement in activity against either TEM-1 or AmpC (Table 1). However, the β-isomers in this series showed a dramatic increase in activity against CcrA. Interestingly, the α-isomers are significantly less active indicating the importance of stereochemistry at the 6-position of these compounds. This is evident by comparing the activities of **14α** and **14β**. The fact that such stereochemical



Scheme 1. Five-step conversion of commercially available 6-(+)-aminopenicillanic acid to the β-isomer of the aldehyde.



Scheme 2. Reagents and conditions: (a) EtMgBr, −78 °C, quench (86%); (b) allyltributyltin/AIBN (79%); (c) O_3, MeOH, Me_2S (69%).



Scheme 3. Reagents and conditions: (a) $RCH{=}PPh_3$, benzene; (b) TFA/CF_3CH_2OH.

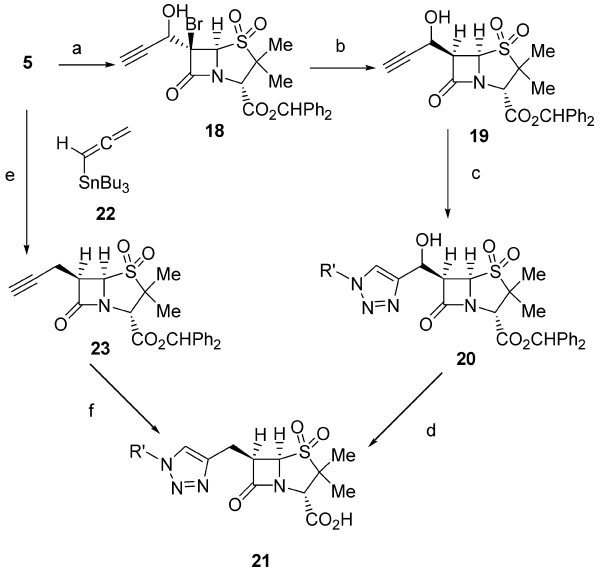
differences have not led to any appreciable change in activity for TEM-1 and AmpC further explains the unique behavior of metalloenzymes.

Table 2 shows the data for the triazole series. The majority of compounds in this series showed improved activity, especially against CcrA and AmpC. Introduction of polar groups to the phenyl substituent in the triazole ring improved TEM-1 activity about 2-fold relative to sulbactam as exemplified by **21c** and **21d**. Further extension of these groups away from the triazole ring via an alkyl chain, as for compound **21f**, decreased the activity against all enzymes. The aniline substituted triazole derivative **21d**[22] showed the best activity profile of all and justified further efforts to find a broad-spectrum β-lactamase inhibitor along these lines.

Table 1. In vitro data for selected compounds of alkenyl series

Compd[a]	R	IC_{50} (μM)[b]		
		TEM-1 (class A)	CcrA (class B)	AmpC (class C)
13α	COMe	8.4	71.0	100.0
14α	CO_2Me	8.6	>100	29.0
15α	CON(OMe)Me	12.0	>100	17.0
16α	CHO	12.0	>100	17.0
13β	COMe	6.5	3.0	40.0
14β	CO_2Me	2.8	1.4	64.0
17β	CN	24.0	3.1	>100
1		1.4	>100	66.0

[a]All compounds shown are (*E*)-isomers.
[b]Determined graphically from six different concentrations of the inhibitor.



Scheme 4. Reagents and conditions: (a) EtMgBr, propargyl aldehyde, THF, −78 °C to rt (61%); (b) Bu_3SnH, AIBN, toluene, 80 °C (71%); (c) $R'N_3$, toluene, reflux; (d) (i) phenylchlorothiono formate, pyr.; (ii) Bu_3SnH, AIBN, toluene (18% for 2 steps); (iii) H_2, Pd–C, 3 h (65%); (e) (i) EtMgBr, −78 °C; (ii) **22**, AIBN, toluene, 16 h (50%); (f) (i) $R'N_3$, toluene (45–70%); (ii) H_2, Pd–C, 3 h (65%).

Further in vitro evaluations of **21d** against β-lactamase-producing organisms indicated that this compound was able to restore the activity of piperacillin to a certain degree, but was less active compared to tazobactam (Table 3). The MIC values of piperacillin in combination with **21d** were improved to 64 μg/mL and 32 μg/mL against AmpC producing *E. coli* (GC2894) and TEM-1 producing *E. coli* (GC2206), respectively.

In summary, a general method was devised to synthesize various alkenyl penam sulfone derivatives. A new method was developed to prepare the propargyl substituted penam sulfone, which was transformed into various triazoles via a cycloaddition protocol. The compounds prepared were evaluated as potential β-lactamase inhibitors. Some of these compounds showed good acitivity against several β-lactamases. Thus, we have demonstrated that allyl and propargyl penam sulfones are valuble intermediates that can be converted into various target molecules having β-lactamase inhibitory activity.

Table 2. In vitro data for selected compounds of triazole series

Compd[a]	R′	IC_{50} (μM)[b]		
		TEM-1 (class A)	CcrA (class B)	AmpC (class C)
21a		1.0	>100	3.4
21b[c]		1.6	4.1	3.0
21c[c]		0.7	2.1	2.3
21d[c]		0.6	1.8	1.4
21e		7.9	22.0	5.2
21f		15.0	24.0	6.6
1		1.4	>100	66.0
2		0.06	>100	48.0

[a]All compounds shown are single regioisomers.
[b]Determined graphically from six different concentrations of the inhibitor.
[c]Substitution is at the *para*-position.

Table 3. In vitro data for **21d** and tazobactam

Compd	MIC (μg/mL)	
	E. coli (GC2894)[b]	*E. coli* (GC2206)[c]
21d[a]	64	32
Tazobactam[a]	16	2
Piperacillin	>64	>64

[a]Piperacillin to inhibitor ratio is 1:1.
[b]AmpC (class C).
[c]TEM-1 (class A).

Acknowledgements

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13. In the ^{1}H NMR spectrum, **6α**-hydrogen shows a coupling constant of 2.0 Hz with the adjacent bridgehead hydrogen whereas **6β**-hydrogen shows a coupling constant of 4.8 Hz. This is consistent with all the other derivatives as well. **4α**: IR 2980, 1800, 1755, 1323, 1179, 1118, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (s, 3H), 1.58 (s, 3H), 3.04 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 19.2$ Hz), 3.21 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 19.2$ Hz), 4.03 (m, 1H), 4.41 (d, 1H, $J = 2.0$ Hz), 4.45 (s, 1H), 6.97 (s, 1H), 7.35 (m, 10H); MS (ES): 442.1 (M + H).
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17. **23**: IR 1760, 1780, 2850, 2950, 3300 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 3H), 1.57 (s, 3H), 2.11 (t, 1H, $J = 2.5$ Hz), 2.78 (dd, 1H, $J_1 = 7.0$ Hz, $J_2 = 2.6$ Hz), 2.82 (dd, 1H, $J_1 = 7.0$ Hz, $J_2 = 2.6$ Hz), 3.86 (m, 1H), 4.49 (s, 1H), 4.54 (d, 1H, $J = 1.8$ Hz), 6.95 (s, 1H), 7.35 (m, 10H); MS (ES): 437.1 (M + H).
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22. **21d**: IR 3400, 2920, 1775, 1630, 1530, 1380, 1320, 1120 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 1.41 (s, 3H), 1.56 (s, 3H), 3.36 (dd, 1H, $J_1 = 7.9$ Hz, $J_2 = 15.5$ Hz), 3.62 (dd, 1H, $J_1 = 10.7$ Hz, $J_2 = 15.5$ Hz), 4.32 (s, 1H), 4.58 (m, 1H), 5.05 (d, 1H, $J = 4.7$ Hz), 6.96 (d, 2H, $J = 8.7$ Hz), 8.21 (s, 1H); MS (ES): 404.2 (M−H).