Chemical Modification of Tazobactam

Synthesis of 2β-[(4-Substituted)-1,2,3-triazol-1-yl]methyl Penicillanic Acid Sulfone Derivatives

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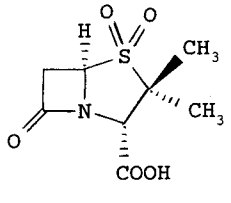
A series of 2β-[(4-substituted)-1,2,3-triazol-1-yl]methyl penicillanic acid sulfones was synthesized as β-lactamase inhibitors. Many of these compounds showed good *in vitro* inhibitory activity against penicillinase, cefotaximase and plasmid-mediated class III TEM enzymes, but exhibited weaker cephalosporinase inhibition. One member in this series—2β-[(4-pyridiniummethyl)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**12a**), when tested in combination with piperacillin, showed excellent synergistic activity against microorganisms producing plasmid-mediated enzymes, but had insufficient activity against microorganisms producing chromosomally mediated class I enzymes.

Introduction of many different penicillins and cephalosporins in the 1960's triggered the spread of plasmid mediated β-lactamases, especially the TEM and SHV types. Broad-spectrum cephalosporins were introduced to prevent hydrolysis by these enzymes. However, extended spectrum mutants of the TEM and SHV enzymes are now reported widely and these variants can hydrolyze broad-spectrum cephalosporins. Resistance to broader-spectrum cephalosporins also can emerge due to the production of the chromosomal class I β-lactamases that typically are inducible in *P. aeruginosa* and in *Enterobacter*, *Citrobacter*, *Morganella*, *Serratia* and *Providencia* species.

The discovery of clavulanic acid and the established clinical efficacy of amoxycillin-clavulanic acid and ticarcillin-clavulanic acid combinations stimulated extensive research in this area[1]. As a result, sulbactam (**1**) was introduced in the market in combination with ampicillin[2]. Following that, YTR-830 (**2**), which was first synthesized by our group in 1983[3,4], has been developed as tazobactam and was introduced in the market recently in combination with piperacillin under the name of tazosin.

Because of the widespread use of third-generation cephalosporins, resistance caused by chromosomally-mediated class I cephalosporinase is increasing rapidly and may pose a threat in future. Over the past ten years there has been a continuous effort to search for new β-lactamase inhibitors[5~10] with an expanded spectrum of activity. One outcome has been the synthesis of BRL-42,715 which is a potent alkylidene penem inhibitor of most bacterial β-lactamases including the class I cephalosporinase[11~13].

Our early attempts at making compounds with the aim of improving biological properties involved the synthesis of 2α-chloromethyl penicillanic acid sulfones[14] and 2α-(1,2,3-triazol-1-yl)methyl penicillanic acid sulfones[15]. However, this modification did not produce compounds with significantly enhanced activity specifically against cephalosporinase. Although tazobactam (YTR-830) showed inhibitory activity against cell-free cephalosporinase, it was ineffective as a synergist against cephalosporinase producing bacterial strain due to poor



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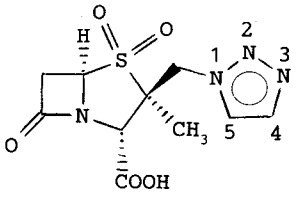

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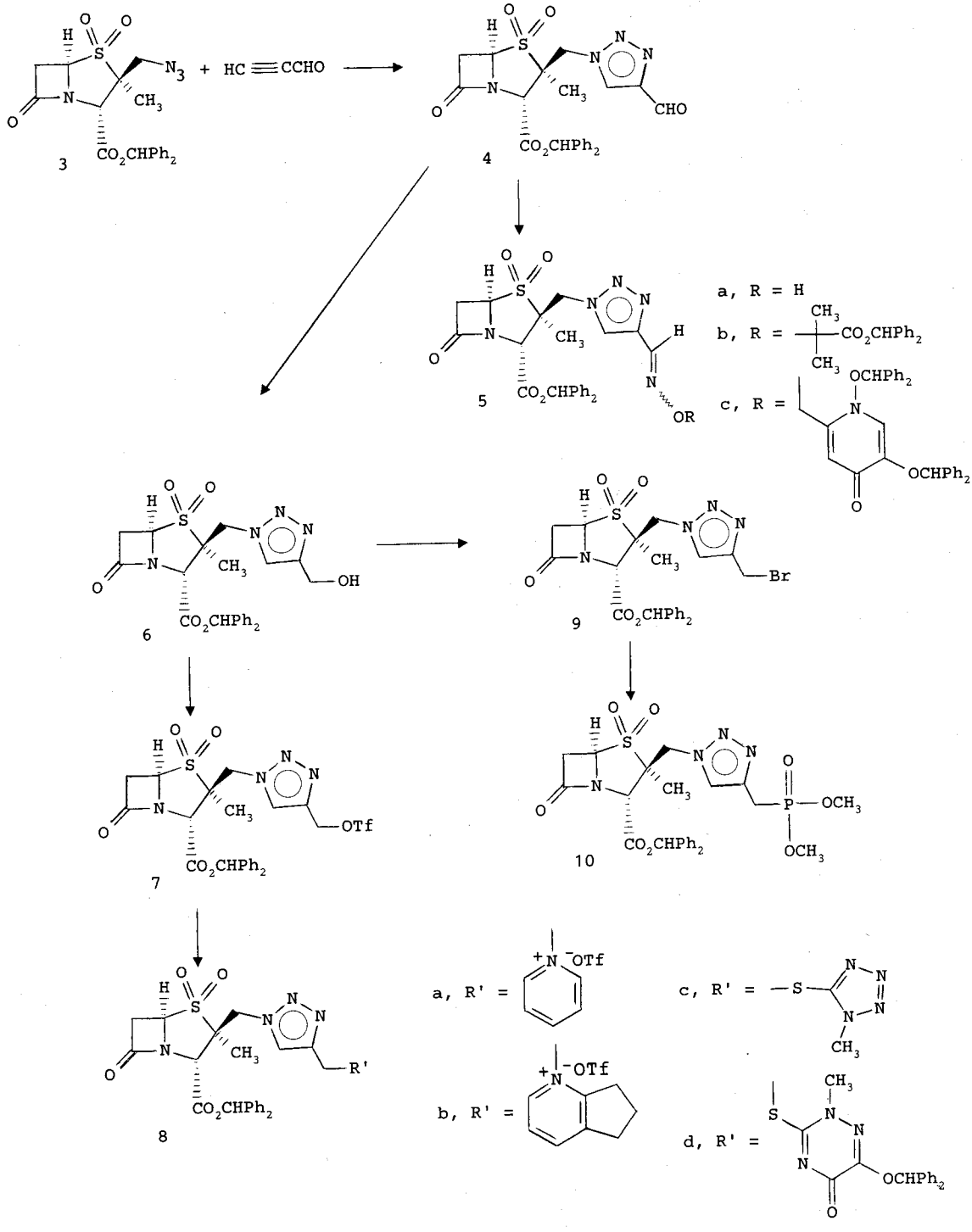
penetration through the bacterial cell wall. The objective of the current study was to modify the 1,2,3-triazole moiety of YTR-830 by introducing various substituents at the C(4) carbon atom with the aim of improving penetration through the cell wall of cephalosporinase producing bacteria. It was anticipated that by introduction of various chemical functions, the physico-chemical properties of the molecule could be altered so that the specific interactions with target enzymes could be achieved and also the penetration could be improved.

We now report the preparation of analogues of (**2**) having varying substituents at C(4) of the triazole ring and their β-lactamase inhibitory activities.

Chemistry

The strategy for the preparation of the title compounds involved the initial introduction of an aldehyde function at the C-4 position of the triazole ring followed by further

Scheme 1. Preparation of 2β-[(4-substituted)-1,2,3-triazol-1-yl]methyl penicillanic acid sulfones.



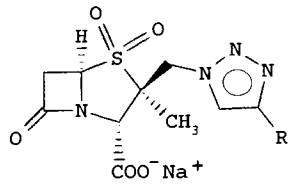
conversion of the aldehyde into various functions. The synthesis of the key compounds **5** (**a**~**c**), **8** (**a**~**d**), and **10** was achieved as outlined in Scheme 1. 2β-Azidomethyl penam sulfone **3**, prepared using standard procedures[16]), was reacted with propargyl aldehyde (obtained by oxidation of propargyl alcohol) to give 2β-[(4-carboxaldehyde-1,2,3-triazol-1-yl)]methyl penam sulfone **4** as the major product. The aldehyde **4** was reacted with appropriate hydroxylamines under standard conditions to provide the oximes **5** (**a**~**c**) in good yield. Reduction of the aldehyde **4** with diisobutylaluminum hydride (DIBAL-H) gave the hydroxymethyl derivative **6**. When the hydroxymethyl derivative **6** was converted to the triflate **7** and treated with excess pyridine, the pyridinium salt **8a** was isolated as the main product. Starting from hydroxymethyl derivative **6**, the corresponding 2,3-cyclopenteno pyridinium salt **8b** was obtained by the same series of reactions. At the same time we were interested in the effect of a thiomethyl group on the biological activity of these compounds, therefore we synthesized the tetrazolylthiomethyl analogue **8c** and triazinylthiomethyl analogue **8d**. The triflate derivative **7** underwent smooth nucleophilic displacement under basic conditions to afford the corresponding thiomethyl analogues **8c** and **8d**.

Conversion of the hydroxymethyl derivative **6** to the bromomethyl derivative **9** was achieved by treatment with N-bromosuccinimide and triphenyl phosphine. Heating of the bromomethyl derivative **9** with excess trimethyl phosphite in acetonitrile gave the dimethoxyphosphinyl methyl derivative **10** in good yield. The compounds were then treated with TFA in the presence of anisole at 0°C to remove the protecting groups and were converted to their sodium salts and chromatographed over Dianion HP-20 column to afford the desired compounds **11** (**a**~**f**). Due to the synthetic expediency the compounds **11a**, **11b** and **11c** were tested as a mixture of syn- and anti-isomers. The compounds **12a** and **12b** were obtained as zwitterions.

Results and Discussion

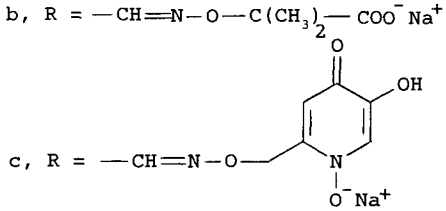
Compounds in series **11** (**a**~**f**) and **12** (**a**~**b**) were tested against cell free β-lactamase preparations and the IC_{50} values are shown in Table 1. Many of these compounds showed good inhibitory activity against penicillinase, cefotaximase and plasmid-mediated class III TEM enzyme, but exhibit weaker cephalosporinase inhibition. Among these, **12a** possesses the most potent β-lactamase inhibitory activity. The compound **11c** bearing the hydroxy pyridone moiety has slightly better activity than tazobactam and other analogues in this series against penicillinase and TEM-enzyme. On the other hand, the chromosomal class I cephalosporinase was not effectively inhibited by any of these inhibitors.

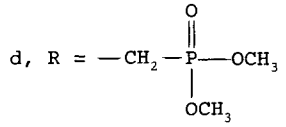
In the cephalosporin class of antibiotics as well as in the monobactam series, increased Gram-negative activity, especially antipseudomonal activity is usually ob-



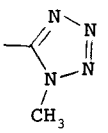
11 a, R = —CH=N—OH

b, R = —CH=N—O—C(CH$_3$)$_2$—COO$^-$ Na$^+$

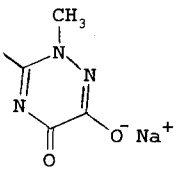


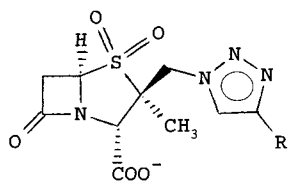


e, R = —CH$_2$—S—

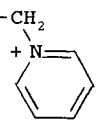


f, R = —CH$_2$—S—

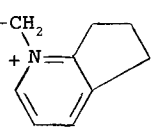




12 a, R =



b, R =



served when the methyl group on the oximino side chain is replaced by an isobutyric acid residue. It was expected that such a replacement might also inhibit the β-lactamase produced by Gram-negative microorganisms including *P. aeruginosa*. In our studies, the incorporation of the isobutyric acid, however, had no effect on the β-lactamase inhibitory activity and thus, compound **11b** failed to protect the antibiotics included in this study against all the strains, particularly against *P. aeruginosa*. In contrast, compound **11a** showed good potentiation of ampicillin activity against *E. coli* and *K. pneumoniae* producing plasmid-mediated β-lactamases, but failed to

Table 1. Inhibitory properties of 2β-[(4-substituted)-1,2,3-triazol-1-yl]methyl penicillanic acid sulfones against isolated β-lactamases.

Compound	IC_{50} (μM) for the β-lactamase			
	Penicillinase (*B. cereus*)	R-TEM (*E. coli*)	Cephalosporinase (*E. cloacae*)	Cefotaximase (*K. pneumoniae*)
Sulbactam	32	1.5	16	—
Tazobactam	0.36	0.025	2.6	0.0028
11a	—	0.01	7.2	0.0069
11b	—	0.0057	4.5	0.0021
11c	0.044	0.0064	4.6	—
11d	0.8	0.02	5.6	0.003
11e	0.7	0.01	6.9	0.002
12a	0.1	0.01	1.6	0.003
12b	—	0.21	8.8	0.03

Table 2. *In vitro* synergy with ampicillin (ABPC) against β-lactamase producing isolates.

Test organisms	MIC (μg/ml)								
	ABPC	+YTR	+**11a**	+**11b**	+**11c**	+**11d**	+**11e**	+**12a**	+**12b**
E. coli TEM-1	>200	1.56	3.13	>200	1.56	100	6.25	1.56	3.13
E. coli TEM-2	>200	50	>200	>200	>200	>200	>200	12.5	>200
E. coli TEM-3	>200	3.13	3.13	>200	(−)	12.5	6.25	3.13	6.25
E. coli TEM-7	>200	3.13	12.5	>200	(−)	50	25	3.13	6.25
E. coli OXA-1	200	50	100	200	6.25	200	200	50	100
E. coli OXA-3	25	1.56	1.56	1.56	(−)	3.13	1.56	1.56	1.56
E. coli SHV-1	>200	3.13	3.13	100	3.13	25	6.25	3.13	6.25
E. coli SHV-5	>200	0.78	1.56	>200	(−)	>200	3.13	1.56	6.25
K. pneumoniae CTX-1	>200	6.25	12.5	>200	>200	>200	50	6.25	12.5
P. aeruginosa 46220	100	6.25	6.25	100	(−)	100	100	50	50
M. morganii 36014	>200	6.25	>200	>200	>200	>200	>200	200	>200
M. morganii 36030	>200	3.13	200	>200	>200	>200	>200	100	200
A. calcoaceticus 450 L	>200	6.25	50	>200	(−)	>200	100	12.5	50
A. calcoaceticus 553 L	>200	(−)	50	>200	(−)	>200	100	12.5	50

Table 3. *In vitro* synergy with ceftazidime (CAZ) against β-lactamase producing isolates.

Test organisms	MIC (μg/ml)								
	CAZ	+YTR	+**11a**	+**11b**	+**11c**	+**11d**	+**11e**	+**12a**	+**12b**
E. coli TEM-3	25	0.2	0.39	6.25	(−)	0.39	0.78	0.39	0.39
E. coli TEM-7	25	0.2	0.39	12.5	(−)	1.56	0.39	0.2	0.2
E. coli OXA-1	0.2	0.2	0.2	0.2	<0.1	0.2	0.2	0.2	0.2
E. coli OXA-3	0.2	<0.1	<0.1	<0.1	(−)	<0.1	<0.1	<0.1	<0.1
E. coli SHV-1	0.2	<0.1	<0.1	0.2	<0.1	0.2	0.2	0.2	<0.1
E. coli SHV-5	200	0.2	0.78	100	(−)	200	1.56	0.39	0.39
K. pneumoniae CTX-1	25	0.39	0.78	12.5	6.25	1.56	0.39	0.39	0.78
P. aeruginosa YP-2	25	12.5	12.5	25	6.25	25	25	12.5	25
P. aeruginosa YP-2-1	100	50	50	100	50	100	100	100	100
M. morganii 36010	200	12.5	50	200	>200	200	200	200	200
M. morganii 36014	25	0.2	3.13	25	25	25	25	3.13	25
M. morganii 36030	12.5	<0.1	1.56	25	25	12.5	12.5	1.56	12.5
A. calcoaceticus 450 L	6.25	(−)	3.13	3.13	(−)	6.25	3.13	1.56	3.13
A. calcoaceticus 553 L	6.25	(−)	3.13	3.13	(−)	6.25	3.13	1.56	3.13

enhance the activity of ampicillin against all class I β-lactamase producing organisms except *P. aeruginosa* 46220 (Table 2). Likewise, when tested in combination with ceftazidime the compound **11a** gave better protection than **11b** against *E. coli* TEM-3, *E. coli* TEM-7, *E. coli* SHV-5, *K. pneumoniae* CTX-1, *P. aeruginosa* YP-2, *M. morganii* 36010, *M. morganii* 36014 and *M. morganii* 36030 (Table 3).

β-Lactam antibiotics variously substituted with catechol or related isosteres show exceptionally good activity against *P. aeruginosa* and other Gram-negative bacteria. It has been found that a ton B-dependent illicit transport route was responsible for the enhanced antibacterial activity[17]. In the monobactam series, introduction of a hydroxy pyridone moiety has led to compounds with improved antipseudomonal activity[18,19]. In order to test the effect of hydroxy pyridone moiety on the chromosomally mediated enzymes produced by *P. aeruginosa*, synthesis of compound **11c** incorporating this function on the oximino side chain was undertaken. Introduction of such a moiety, however, had no effect on the cell free cephalosporinase isolated from *P. aeruginosa* (Table 1), but was somewhat more active against penicillinase and TEM enzyme. A combination

Table 4. *In vitro* synergy with ceftriaxone (CTRX) against β-lactamase producing isolates.

Test organisms	MIC (μg/ml)							
	CTRX	+YTR	+**11a**	+**11b**	+**11c**	+**11e**	+**12a**	+**12b**
E. coli TEM-3	6.25	<0.1	<0.1	0.78	(−)	<0.1	<0.1	<0.1
E. coli TEM-7	0.2	<0.1	<0.1	<0.1	(−)	<0.1	<0.1	<0.1
E. coli SHV-5	12.5	<0.1	<0.1	6.25	(−)	<0.1	<0.1	<0.1
K. pneumoniae CTX-1	50	<0.1	<0.1	3.13	1.56	<0.1	<0.1	<0.1
P. aeruginosa 46220	3.13	0.78	0.78	0.78	(−)	1.56	0.78	0.78
P. aeruginosa 46001	25	25	25	25	50	50	12.5	12.5
P. aeruginosa 46002	6.25	6.25	6.25	6.25	6.25	6.25	3.13	3.13
P. aeruginosa 46025	25	3.13	3.13	25	50	12.5	25	12.5
M. morganii 36010	50	0.78	25	50	100	50	25	25
M. morganii 36014	3.13	<0.1	0.2	1.56	1.56	3.13	<0.1	1.56
M. morganii 36030	1.56	<0.1	0.2	1.56	1.56	1.56	<0.1	0.78
A. calcoaceticus 450 L	12.5	(−)	12.5	12.5	(−)	12.5	3.13	12.5
A. calcoaceticus 553 L	12.5	(−)	12.5	12.5	(−)	25	3.13	12.5

Table 5. *In vitro* synergy with piperacillin (PIPC) against β-lactamase producing isolates.

Test organisms	MIC (μg/ml)			
	PIPC	+YTR	+**12a**	+**12b**
S. aureus CT-10	200	50	50	50
S. aureus HL-1185	200	25	12.5	50
S. aureus 54K	25	0.78	0.78	0.78
S. aureus 80K	12.5	0.39	0.39	0.39
E. coli TEM-1	100	0.78	0.78	0.78
E. coli TEM-2	>200	12.5	1.56	50
E. coli TEM-3	200	1.56	1.56	1.56
E. coli TEM-7	200	0.78	0.78	0.78
E. coli OXA-1	12.5	3.13	3.13	6.25
E. coli OXA-3	3.13	0.78	0.78	0.78
E. coli SHV-1	12.5	1.56	1.56	0.78
E. coli SHV-5	200	<0.1	0.2	0.78
K. pneumoniae 336L	100	3.13	3.13	3.13
K. pneumoniae CTX-1	200	6.25	6.25	6.25
S. marcescens 200L	50	0.39	0.39	0.39
S. marcescens CT-98	200	25	25	50
P. vulgaris CT-106	100	0.78	0.78	3.13
C. freundii 2046E	25	0.39	0.39	0.39
C. freundii CT-26	>200	12.5	6.25	100
E. cloacae P99	100	12.5	12.5	25
E. cloacae 212L	>200	50	3.13	100
P. aeruginosa 46220	0.78	0.39	0.39	0.39
P. aeruginosa YP-2	50	6.25	25	25
P. aeruginosa YP-2-1	200	50	100	200

of the hydroxy pyridone analogue, **11c** either with ampicillin (Table 2) or with ceftazidime (Table 3) failed to demonstrate a remarkable synergistic effect against *E. coli*. This could be due to the fact that this compound cannot penetrate the outer cell membrane of bacteria. When tested in combination with ceftriaxone (CTRX) none of the synthesized compounds was superior to YTR (Table 4) against all the bacterial strains tested.

The introduction of a positive charge at 3'-position of the cephalosporin nucleus usually gives increased antibacterial activity against Gram-negative organisms including *P. aeruginosa*. In order to see the effect on synergistic activity of introducing a positively charged pyridinium group on the triazole ring, compounds **12a** and **12b** were synthesized. The good *in vitro* activity of **12a** against the cephalosporinase was not evident in the synergistic effect, however. The derivative **12a** showed reduced synergistic activity against the enzymes produced by *S. aureus* HL-1185, *E. coli* TEM-2, *C. freundii* CT-26, *E. cloacae* 212 L, but synergistic activity against all other β-lactamases was similar to that seen with the uncharged analogue tazobactam (Table 5).

Conclusion

Despite the effort to introduce various functional groups at the C-4 position of the triazole ring of tazobactam with the hope of getting a well-balanced β-lactamase inhibitor including the class I cephalosporinase, we failed to find a compound having sufficient activity against chromosomally mediated class I enzymes. In this series, the compound **12a** when tested in combination with piperacillin showed excellent activity against microorganisms producing plasmid-mediated enzymes but had insufficient activity against microorganisms producing chromosomally mediated class I enzymes.

Experimental

General Comments

All column chromatographic purifications were accomplished on silica gel 60 (E. Merck, 230~400 mesh) with the appropriate solvent gradients. TLC was done on commercial silica gel plates (Analtech) containing calcium sulfate binder and fluorescent indicator. ^{1}H NMR spectra were determined with a Bruker AC-200-F (200 MHz) spectrometer in appropriate deuterated solvents and are expressed in ppm downfield from TMS (internal standard).

β-Lactamase inhibition studies were carried out on isolated enzyme preparations by spectrophotometric monitoring of hydrolysis of nitrocefin in the presence and absence of test compound. MICs were determined by the agar dilution method, followed by addition of inhibitor (5 μg/ml) and organism (approx. 2×10^6 cfu/ml). MIC values were recorded after incubation at 37°C for 18 hours. The growth of microorganisms was observed to determine the minimal inhibitory concentration (MIC) for rendering the inoculated medium free from turbidity. Tazobactam was used as the reference compound and was synthesized in our laboratory.

Benzhydryl 2β-[(4-Carboxaldehyde)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**4**)

Benzhydryl 2β-azidomethyl-6,6-dihydropenicillanate-1,1-dioxide (6.0 g, 0.0136 mol) was dissolved in dimethoxyethane (20 ml). To this solution, freshly prepared and distilled propargyl aldehyde (2.0 g) was added and the mixture was heated at 50°C for 18 hours. The precipitated solid was filtered off and washed with ether, air dried. The desired product **4** was obtained as an off-white solid (3.7 g, 55%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.07 (3H, s), 3.45~3.69 (2H, m), 4.60~4.68 (1H, m), 4.70 (1H, s), 5.12 (2H, ABq), 7.05 (1H, s), 7.35~7.49 (10H, m), 8.28 (1H, s), 10.16 (1H, s).

Sodium 2β-[(4-Oximino)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**11a**)

To a suspension of the ester **4**, (495 mg, 1 mmol) in anisole at 0°C, TFA (770 μl, 10 mmol) was added dropwise and the mixture was stirred at 0°C for 1.5 hours and at room temp. for 0.5 hour. After addition of isopropyl ether (6 ml), the mixture was stirred at 0°C for 10 minutes. The white precipitated solid was collected by filtration and vacuum dried (323 mg). The acid (323 mg, 0.985 mmol) thus obtained was suspended in water (15 ml) and sodium bicarbonate (248 mg, 2.95 mmol) was added. To this solution hydroxylamine hydrochloride (70 mg, 1 mmol) was added and the mixture was stirred at room temperature for 18 hours. The mixture was concentrated and purified by passing through a Dianion HP-20 column starting with water and finishing with water - acetone mixture (9 : 1) as eluant. The product **11a** was obtained as a white floppy amorphous powder (95 mg, 26% overall yield) as a mixture of syn- and anti-isomers.

^{1}H NMR (200 MHz, D_2O) for one isomer δ 1.45 (3H, s), 3.43~3.76 (2H, ABX system), 4.50 (1H, s), 5.02~5.06 (1H, m), 5.29 (2H, ABq), 7.83 (1H, s), 8.85 (1H, s).

^{1}H NMR (200 MHz, D_2O) for other isomer δ 1.45 (3H, s), 3.43~3.76 (2H, ABX system), 4.49 (1H, s), 5.02~5.06 (1H, m), 5.26 (2H, ABq), 8.32 (1H, s), 8.39 (1H, s).

Benzhydryl 2β-[4-(1-Methyl-1-diphenylmethyloxy carbonyl ethoxyimino)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**5b**)

To a solution of N-[(1-methyl-1-diphenylmethyloxy carbonyl)ethoxy] phthalimide (539 mg, 1.299 mmol) in dry THF at 0°C, anhydrous hydrazine (41 μl, 1.299 mmol) was added. The mixture was stirred at 0°C for 15

minutes and then at room temperature for 2.5 hours. The solvent was partially removed under reduced pressure; the insoluble material was removed by filtration through a small bed of Celite. The filtrate was concentrated to dryness and the residue was dissolved in methylene chloride (5 ml), cooled to 0°C and the insoluble material was removed by filtration. To the filtrate, the aldehyde **4** (638 mg, 1.299 mmol) was added and the mixture was stirred at room temperature for 40 hours. After concentration, the product was chromatographed over a silica gel column using ethyl acetate - benzene (3 : 7) mixture as eluant. The title compound was obtained as a foam (475 mg, 48.3%) as a mixture of syn- and anti-isomer in the ratio of (*ca.* 1 : 1).

^{1}H NMR (200 MHz, $CDCl_3$) for one isomer δ 1.04 (3H, s), 1.64 (3H, s), 1.68 (3H, s), 3.52~3.56 (2H, m), 4.59~4.63 (1H, m), 4.68 (1H, s), 5.03 (2H, ABq), 6.87 (1H, s), 7.00 (1H, s), 7.20~7.42 (20 H, m), 7.73 (1H, s), 8.55 (1H, s).

^{1}H NMR (200 MHz, $CDCl_3$) for other isomer δ 1.04 (3H, s), 1.58 (3H, s), 1.60 (3H, s), 3.52~3.56 (2H, m), 4.59~4.63 (1H, m), 4.70 (1H, s), 5.00 (2H, s), 6.90 (1H, s), 7.00 (1H, s), 7.20~7.42 (20 H, m), 7.66 (1H, s), 8.26 (1H, s).

2β-[[4-(1-Carboxy-1-methyl ethoxyimino)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanic Acid 1,1-dioxide, Disodium Salt (**11b**)

To a solution of the ester **5b**, (460 mg, 0.604 mmol) in anisole (0.6 ml) at 0°C, TFA (558 μl, 7.25 mmol) was added over 15 minutes. The mixture was stirred at 0°C for 1 hour and then at room temperature for 30 minutes; isopropyl ether (6 ml) was added and the suspension was stirred at 0°C for 10 minutes. The white solid was collected by filtration, washed with isopropyl ether and dried under vacuum (229 mg). To the acid, an aqueous solution of $NaHCO_3$ (179 mg dissolved in 3 ml of water) was added and the suspension was stirred at 0°C for 1 hour. The product was purified through a Dianion HP-20 column using water and finally water - acetone mixture (95 : 5) as eluant. After freeze-drying the product was obtained as a mixture of syn- and anti-isomer (3 : 2) as a light yellow amorphous powder (95 mg, 33.2%).

^{1}H NMR (200 MHz, D_2O) for one isomer δ 1.50 (6H, s), 1.54 (3H, s), 3.43~3.76 (2H, ABX system), 4.50 (1H, s), 5.03~5.05 (1H, m), 5.29 (2H, ABq), 7.80 (1H, s), 8.78 (1H, s).

^{1}H NMR (200 MHz, D_2O) for other isomer δ 1.44 (6H, s), 1.53 (3H, s), 3.43~3.76 (2H, ABX system), 4.49 (1H, s), 5.03~5.05 (1H, m), 5.26 (2H, ABq), 8.29 (1H, s), 8.44 (1H, s).

Benzhydryl 2β-[[4-(1,5-Dibenzhydryloxy-4-pyridone-2-yl-methoxyimino)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**5c**)

To an ice-cooled solution of 2-phthalimidooxymethyl-1,5-dibenzhydryloxy-4-pyridone (1.30 g, 2.05 mmol) in dry THF (4 ml) was added anhydrous hydrazine (65 μl, 2.06 mmol). The resulting mixture was stirred at 0°C for 1 hour and at room temp. for 1.5 hours. The precipitated solid was removed by filtration and the filtrate was concentrated to dryness. The residue was dissolved in methylene chloride (8 ml) and the solution was cooled to 0°C. The insoluble material was removed by filtration through a bed of Celite. The filtrate was diluted with methylene chloride to make the volume of about 20 ml. To this solution the aldehyde **4** (984 mg, 2.05 mmol) was added and the mixture was stirred at room temperature for 18 hours. After removal of the solvent the crude product was purified over a silica gel column by using ethyl acetate - hexane (7 : 3) as eluant to give the title compound **5c** as an oil (1.22 g, 62%). The product was obtained as a mixture of syn- and anti-isomer in the ratio of (1 : 1).

^{1}H NMR (200 MHz, $CDCl_3$) for one isomer δ 1.05 (3H, s), 3.48~3.58 (2H, m), 4.55~4.63 (1H, m), 4.66 (1H, s), 4.80 (2H, ABq), 5.00 (2H, ABq), 5.83 (1H, s), 6.10 (1H, s), 6.82 (1H, s), 7.0 (1H, s), 7.16~7.50 (30H, m), 7.90 (1H, s), 8.26 (1H, s).

^{1}H NMR (200 MHz, $CDCl_3$) for other isomer δ 1.05 (3H, s), 3.48~3.58 (2H, m), 4.55~4.63 (1H, m), 4.66 (1H, s), 4.80 (2H, ABq), 5.06 (2H, ABq), 5.90 (1H, s), 6.09 (1H, s), 6.80 (1H, s), 7.00 (1H, s), 7.16~ 7.50 (30H, m), 7.72 (1H, s), 8.16 (1H, s).

Sodium 2β-[[4-(1,5-Dihydroxy-4-pyridone-2-yl methoxyimino)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**11c**)

To a solution of the compound **5c** (500 mg, 0.509 mmol) in dry anisole (0.6 ml) at -5°C, TFA (0.588 ml, 7.64 mmol) was added dropwise. The mixture was stirred at 0°C for 1 hour and at room temp. for 15 minutes; isopropyl ether (5 ml) was added and the suspension was stirred at 0°C for 10 minutes. The precipitated solid was collected by filtration and dried under vacuum. The acid thus obtained was dissolved in an aqueous solution of sodium bicarbonate (80 mg dissolved in 2 ml of water). After stirring at 0°C for 1 hour, the crude product was purified by column chromatography on Dianion HP-20 column. After freeze drying the desired compound was obtained as a floppy beige solid as a mixture of syn- and anti-isomers (88 mg, 26% overall yield).

^{1}H NMR (200 MHz, D_2O) for one isomer δ 1.40 (3H, s), 3.39~3.71 (2H, ABX system), 4.46 (1H, s), 4.99~5.01 (1H, m), 5.08~5.38 (4H, 2 overlapping ABq), 6.55 (1H, s), 7.61 (1H, s), 7.85 (1H, s), 8.81 (1H, s).

^{1}H NMR (200 MHz, D_2O) for other isomer δ 1.40 (3H, s), 3.39~3.71 (2H, ABX system), 4.46 (1H, s), 4.99~5.01 (1H, m), 5.08~5.38 (4H, 2 overlapping ABq), 6.67 (1H, s), 7.61 (1H, s), 8.38 (1H, s), 8.39 (1H, s).

Benzhydryl 2β-[[4-(Dimethoxyphosphinylmethyl)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**10**)

To a solution of the alcohol **6** (200 mg, 0.403 mmol)

in acetonitrile (6 ml), triphenyl phosphine (105 mg, 0.403 mmol) and N-bromosuccinimide (71.7 mg, 0.403 mmol) were added and the reaction mixture was stirred at room temperature for 3 hours. After removal of the solvent, the product was purified over a silica gel column. Elution of the column with benzene - ethyl acetate (7 : 3) as eluant gave benzhydryl 2β-[(4-bromomethyl)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**9**) as a white foam (64%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (3H, s), 3.42 ~ 3.68 (2H, ABX system), 4.55 (2H, s), 4.60 ~ 4.65 (1H, m), 4.63 (1H, s), 5.04 (2H, ABq, J = 15.0 and 19.5 Hz), 7.00 (1H, s), 7.28 ~ 7.45 (10H, m), 7.75 (1H, s).

To a solution of the bromomethyl from the above step, **9** (400 mg, 0.715 mmol) in acetonitrile (1 ml), trimethyl phosphite (843 μl, 7.15 mmol) was added and the mixture was heated at 80°C for 2 hours 15 minutes. Volatile materials were removed under reduced pressure. To the oily residue a mixture of hexane - ether (1 : 1) was added. The product thus obtained was pure enough for use in the next step (350 mg, 83.5% yield).

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.06 (3H, s), 3.28 (1H, br, s), 3.38 (1H, br s), 3.40 ~ 3.62 (2H, ABX system), 3.71 (3H, d, J = 3.9 Hz), 3.76 (3H, d, J = 3.9 Hz); 4.60 (1H, dd, J = 1.5 and 2.4 Hz), 4.64 (1H, s), 5.04 (2H, ABq, J = 15.1 and 16.6 Hz), 7.00 (1H, s), 7.25 ~ 7.44 (10H, m), 7.75 (1H, d, J = 2.4 Hz).

Sodium 2β-[[4-(Dimethoxyphosphinylmethyl)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**11d**)

To a solution of the ester **10** (340 mg, 0.578 mmol) in anisole (500 μl) at −5°C, TFA (623 μl, 8.09 mmol) was added. The mixture was stirred at −5°C for 1 hour. Isopropyl ether (6 ml) was added and stirred at 0°C for 15 minutes. The precipitated solid was collected by filtration and washed several times with dry ether. The acid (147 mg, 0.348 mmol) was suspended in 2 ml of water, 30 mg (0.348 mmol) of sodium bicarbonate was added and stirred at room temperature for 1 hour. The crude product was purified over a HP-20 column using water - acetone (9 : 1) mixture as eluant. The title compound **11d** was obtained as an off-white fluffy mass (90 mg).

^{1}H NMR (200 MHz, D_2O) δ 1.43 (3H, s), 3.46 (1H, dd, J = 1.35 and 16.5 Hz), 3.50 (1H, s), 3.60 (1H, s), 3.70 (1H, dd, J = 4.23 and 16.5 Hz), 3.75 (3H, s), 3.80 (3H, s), 4.46 (1H, s), 5.02 (1H, dd, J = 1.3 and 2.67 Hz), 5.22 (2H, ABq, J = 15.3 and 40.0 Hz), 8.10 (1H, d, J = 2.7 Hz).

Benzhydryl 2β-[[4-(1-Methyl-1H-tetrazol-5-ylthiomethyl)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**8c**)

To a solution of the alcohol **6** (300 mg, 0.605 mmol) in methylene chloride (5 ml) at −40°C, triethylamine (100 μl, 0.725 mmol) and trifluoromethane sulfonic anhydride (122 μl, 0.725 mmol) were added and the mixture was stirred at −40°C for 1 hour. To this mixture a solution of 1-methyl-5-mercapto-1,2,3,4-tetrazole (84 mg, 0.725 mmol) dissolved in a mixture of methylene chloride (0.5 ml) and triethylamine (100 μl, 0.725 mmol) were added. After the addition was over, the mixture was stirred at 0°C for 2.5 hours. After usual workup, the product was purified over a silica gel column using ethyl acetate - hexane mixture (4 : 1) as eluant. The product was obatined as a white foam (133 mg, 37%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.03 (3H, s), 3.40 ~ 3.63 (2H, m), 3.86 (3H, s), 4.56 ~ 4.59 (1H, m), 4.60 (1H, s), 4.62 (2H, s), 4.97 (2H, s), 6.99 (1H, s), 7.27 ~ 7.45 (10H, m), 7.80 (1H, s).

Sodium 2β-[[4-(1-Methyl-1H-tetrazol-5-ylthiomethyl)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**11e**)

The benzhydryl ester (**8c**, 260 mg, 0.438 mmol) was dissolved in 500 μl of anisole and cooled to −5°C. To this solution TFA (505 μl, 6.56 mmol) was added and the mixture was stirred at −5°C for 1 hour. To this mixture isopropyl ether was added, the precipitated solid was collected by filtration and washed thoroughly with ether. The crude acid (202 mg) was crystallized from acetone - ether (138 mg).

The acid (138 mg, 0.322 mmol) was suspended in 2 ml of water, 28 mg (0.322 mmol) of $NaHCO_3$ was added. After stirring at room temperature for 1 hour, the mixture was filtered through a small bed of Celite, then purified through a HP-20 column using water - acetone mixture (9 : 1) as eluant. After freeze-drying the product was obtained as white fluffy solid (65 mg, 33%).

^{1}H NMR (200 MHz, D_2O) δ 1.31 (3H, s), 3.42 (1H, dd, J = 1.5 and 16.7 Hz), 3.67 (1H, dd, J = 4.3 and 16.7 Hz), 3.92 (3H, s), 4.41 (1H, s), 4.56 (2H, s), 4.97 (1H, dd, J = 1.5 and 4.3 Hz), 5.14 (2H, ABq, J = 15.3 and 41.2 Hz), 8.00 (1H, s).

Benzhydryl 2β-[[4-(2,5-Dihydro-6-benzhydryloxy-2-methyl-5-oxo-as-triazin-3-ylthiomethyl)]-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**8d**)

To a solution of the alcohol **6**, (250 mg, 0.504 mmol) in methylene chloride (6 ml) at −40°C, triethylamine (84 μl, 0.604 mmol) and trifluoromethane sulfonic anhydride (101 μl, 0.604 mmol) were added and the mixture was stirred at −40°C for 1 hour. To this mixture, 2,5-dihydro-6-benzhydryloxy-3-mercapto-2-methyl-5-oxo-as-triazin (197 mg, 0.604 mmol) was added followed by potassium t-butoxide (67 mg, 0.604 mmol). The reaction mixture was stirred at −40°C for 1 hour and then at room temperature for 1 hour. Solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (25 ml) and washed with brine (15 ml). After drying over anhydrous Na_2SO_4, the crude product was purified over a silica gel column using ethyl acetate - hexane mixture as eluant (151 mg, 37.2%). This product was directly used for deprotection.

Sodium 2β-[[4-(2,5-Dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-ylthiomethyl)]-1,2,3-triazol-1-yl]-methyl-6,6-dihydropenicillanate 1,1-dioxide (**11f**)

The benzhydryl ester from the previous step **8d** (322 mg, 0.400 mmol) was dissolved in 700 μl of anisole and cooled to 0°C. To this solution, TFA (617 μl, 8.0 mmol) was added and the mixture was stirred at 0°C for 1 hour; 6 ml of isopropyl ether was added, the precipitated solid was collected by filtration and washed thoroughly with ether. The crude acid (178 mg, 0.377 mmol) was taken in (2 ml) of water and 32 mg (0.377 mmol) of $NaHCO_3$ was added and stirred for a while. The solution was freeze-dried to give a mass (160 mg) which was purified by reverse phase preparative TLC using acetonitrile - water (7 : 3). The mass of the product was 31 mg, which was repurified by passing through a HP-20 column (20 mg, 10% yield).

^{1}H NMR (200 MHz, D_2O) δ 1.37 (3H, s), 3.42 (1H, dd, J=1.4 and 16.9 Hz), 3.57 (3H, s), 3.64 (1H, dd, J=3.7 and 16.9 Hz), 4.42 (1H, s), 4.47 (2H, ABq, J=14.8 and 24.8 Hz); 4.90~4.96 (1H, m), 5.14 (2H, ABq, J=15.0 and 40.0 Hz), 8.03 (1H, s).

Benzhydryl 2β-[(4-Hydroxymethyl)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**6**)

To a solution of the aldehyde (**4**, 1.976 g, 5 mmol) in 60 ml of dry THF cooled to −60°C was added DIBAL-H (4.10 ml, 4.1 mmol, 1.0 M solution in DCM) and the reaction mixture was stirred at −60°C for 2.5 hours, brine (10 ml) was added. The organic layer was separated out and diluted with ethyl acetate (60 ml). The organic layer was washed with brine (15 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified over a silica gel column using ethyl acetate as eluant to give the title compound as a white foam (1.55 g, 78%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (3H, s), 2.55 (1H, br s), 3.42~3.54 (2H, ABX system), 4.60~4.67 (1H, m), 4.67 (1H, s), 4.77 (2H, s), 5.02 (2H, ABq), 7.00 (1H, s), 7.28~7.43 (10H, m), 7.69 (1H, s).

2β-[(4-Pyridiniummethyl)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**12a**)

The alcohol **6**, (200 mg, 0.403 mmol) was dissolved in dry DCM (6 ml) and cooled to −40°C, pyridine (0.651 ml, 8.06 mmol) and trifluoromethane sulfonic anhydride (0.237 ml, 1.41 mmol) were added. The reaction mixture was stirred at −40°C for 1.5 hours and then at room temperature for 0.5 hour. Solvent was partially removed under reduced pressure to make a volume of about 2 ml, dry ether (15 ml) was added. The ether layer was decanted off; the oily residue was dissolved in ethyl acetate (30 ml) and washed with aqueous citric acid (10% solution, 12 ml), then with brine (10 ml). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give the triflate salt. This product was subjected to deprotection without further purification.

The triflate salt from the previous step (340 mg, 0.480 mmol) was dissolved in 0.5 ml of anisole and cooled to 0°C. To this solution TFA (0.555 ml, 7.2 mmol) was added and the mixture was stirred at 0°C for 1 hour 15 minutes; 6 ml of isopropyl ether was added; the precipitated solid was collected by filtration and washed thoroughly with ether. The acid (240 mg, 0.475 mmol) was suspended in 2 ml of water, sodium bicarbonate (40 mg, 0.476 mmol) was added and stirred for 15 minutes. The product was purified over a HP-20 column. The column was eluted initially with water and finally with water - acetone (9 : 1). The title compound was obtained as an orange floppy mass (65 mg, 35% overall yield).

^{1}H NMR (200 MHz, D_2O) δ 1.42 (3H, s), 3.57 (2H, ABX system), 4.48 (1H, s), 5.01~5.03 (1H, m), 5.26 (2H, ABq), 6.01 (2H, s), 8.12 (2H, t), 8.45 (1H, s), 8.61 (1H, t), 8.96 (2H, d).

Compound **12b** was prepared from benzhydryl 2β-[(4-hydroxymethyl)-1,2,3-triazol-1-yl]methyl-6,6-dihydropenicillanate 1,1-dioxide (**6**) and 2,3-cyclopenteno pyridine by a procedure similar to that described for **12a**.

^{1}H NMR (200 MHz, D_2O) for **12b** δ 1.43 (3H, s), 2.26~2.39 (2H, m), 3.20 (1H, t), 3.38 (1H, t), 3.43 (1H, dd), 3.70 (1H, dd), 4.47 (1H, s), 5.00~5.02 (1H, m), 5.25 (2H, ABq), 5.89 (2H, s), 7.80 (1H, t), 8.33 (1H, d), 8.40 (1H, s), 8.61 (1H, d).

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