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# Synthesis and biological evaluation of penam sulfones as inhibitors of β-lactamases

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**Abstract**—The chemical synthesis of a series of new penam sulfone derivatives bearing a 2β-substituted-oxyimino and -hydrazone substituents, their β-lactamase inhibitory properties against selected enzymes representing class A and C β-lactamases are reported. The oxime containing penam sulfones strongly inhibited the *Escherichia coli* TEM-1 and *Klebsiella pneumoniae* cefotaximase (CTX-1) enzymes, but moderately inhibited the *Pseudomonas aeruginosa* 46012 cephalosporinase; while the 2β-substituted-hydrazone derivatives were generally less active against these enzymes. Furthermore, most of the inhibitors enhanced the antibacterial activities of piperacillin (PIP) and ceftazidime (CAZ) particularly against TEM-1 and CTX-1 producing bacterial strains.


## 1. Introduction

The extensive use of β-lactam antibiotics in hospitals and in the community has created major resistance problems world wide, leading to increased morbidity, mortality, and health-care costs. The most common form of such resistance is the production of β-lactamases, which hydrolyze the β-lactam ring of β-lactam antibiotics. Hence, the production of β-lactamases remains the main mechanism for bacterial resistance to β-lactam antibiotics.[1,2] The hydrolytic destruction of the β-lactam antibiotics may be either serine enzyme (classes A, C, and D) or metallo-β-lactamases (class B) catalyzed.[3] The classification of the enzymes into these molecular classes is based on their primary structures and sequence homology.[3–5] Both class A and C enzymes continue to be of clinical importance because of their production in strains of Enterobacteriaceae and *Pseudomonas aeruginosa*, selected by the extensive use of the newer-generation cephalosporins.[1,3,6–8] A highly effective and proven antibacterial strategy for overcoming β-lactamase-mediated resistance to β-lactam antibiotics is the use of β-lactam–β-lactamase inhibitor combinations.[9–11] Established inhibitors of the serine β-lactamases including clavulanic acid **1**, sulbactam **2a**, and tazobactam (TAZ) **2b**, (Fig. 1) are useful clinically,[11] particularly against mixed infections. These inhibitors target the most clinically relevant class A β-lactamase producing microorganisms. However, the class C enzymes (AmpC, cephalosporinases) are of profound

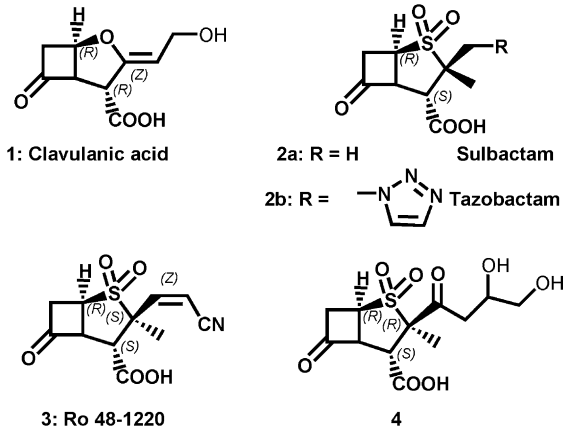


**Figure 1.** Structures of β-lactamase inhibitors.

*Keywords*: Antibacterial activity; β-Lactamases; β-Lactamase inhibitors; Penam sulfones.
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clinical importance because they are not significantly inhibited by any of the clinically used β-lactamase inhibitors.[12,13] Moreover, class C enzymes naturally have broad-spectrum of action and can hydrolyze 'β-lactamase resistant' β-lactams, such as the third-generation cephalosporins.[11] Furthermore, β-lactam-based inhibitors and newer cephalosporins can upregulate the expression of class C β-lactamases, thus resulting in the selection of mutants that hyperproduce chromosomally encoded AmpC β-lactamase through stable derepression.[14–16] Both chromosomal and plasmid-borne class C β-lactamases are reported to be present in 10–50% of clinical isolates of *Citrobacter freundii*, *Entrobacter cloacae*, *Serratia marcescens*, and *P. aeruginosa*.[17] Plasmid-encoded AmpC β-lactamases have also been described in clinical isolates of *Klebsiella* spp. and *Escherichia coli*.[18]

Scientists from Hoffmann La-Roche reported the synthesis of a series of 2β-alkenyl penam sulfones that possess the ability to simultaneously inactivate both class A pencillinases as well as class C cephalosporinases.[19] A compound Ro 48-1220 (**3**, Fig. 1) from this series showed best synergy in combination with ceftriaxone against resistant microorganisms. The increased spectrum of activity of Ro 48-1220 was not associated with increased affinity for, or enhanced reactivity with the inhibited enzyme, but rather correlated to the slow deacylation of the inhibited enzyme.[19] This was ascribed to the presence of the 2β-substituted C=C double bond side chain of the penam sulfone **3**. The clinical situation now dictates that second-generation β-lactamase inhibitors capable of encompassing both class A and class C β-lactamases would combat emerging resistance and provide a vital addition to the antibacterial armory. We have previously reported 2β-acyl penam sulfones, exemplified by **4** with extended-spectrum β-lactamase inhibitory activities.[20] In continuation of our search for new broad-spectrum β-lactamase inhibitors, we reasoned that the introduction of the 2β-substituted C=N double bond should produce analogues with strong β-lactamase inhibitory activity. We hereby report the syntheses, β-lactamase inhibitory and synergistic activities of a series of 2β-substituted penam sulfone derivatives **17a**–**n** and **18a**–**f** against resistant microorganisms in combination with clinically used β-lactam antibiotics.

## 2. Chemistry

The key intermediate, benzyhydryl-2β-formy1-6,6-dihydropenicillanate-1,1-dioxide **12** utilized in this work was synthesized starting from the commercially available 6-aminopencillanic acid **5**, as shown in Scheme 1. The azetidinone derivative **6** was prepared according to the literature methods.[21,22] Compound **6** was reacted with chloroacetic acid in the presence of silver acetate to give an inseparable mixture of benzhydry1-2β-(chloroacetoxy)methyl-6,6-dihydropenicillanate **7** and benzhydry1-3-(chloroacetoxy)-3-methyl-6,6-dihydrocephalosporinate **8**. The mixture of **7** and **8** was oxidized with potassium permanganate in glacial acetic acid to give the corresponding sulfones **9** and **10**, respectively. Column chromatographic purification gave pure benzyhydryl-2β-(chloroacetoxy)methyl-6,6-dihydropencillanate-1,1-dioxide, **9**. Compound **9** was treated with thiourea in ethanol to give benzhydryl-2β-hydroxymethyl-6,6-dihydropencillanate-1,1-dioxide **11**, which was oxidized with pyridinium chlorochromate (PCC) in dichloromethane to give the key intermediate benzyhydryl-2β-formy1-6,6-dihydropenicillanate-1,1-dioxide **12** in 76% yield. Further chemical transformation by the reaction of compound **12** with various alkoxylamines and substituted hydrazines gave the corresponding oxime **13a**–**m** and hydrazone **14a**–**f** derivatives, respectively (Table 1).

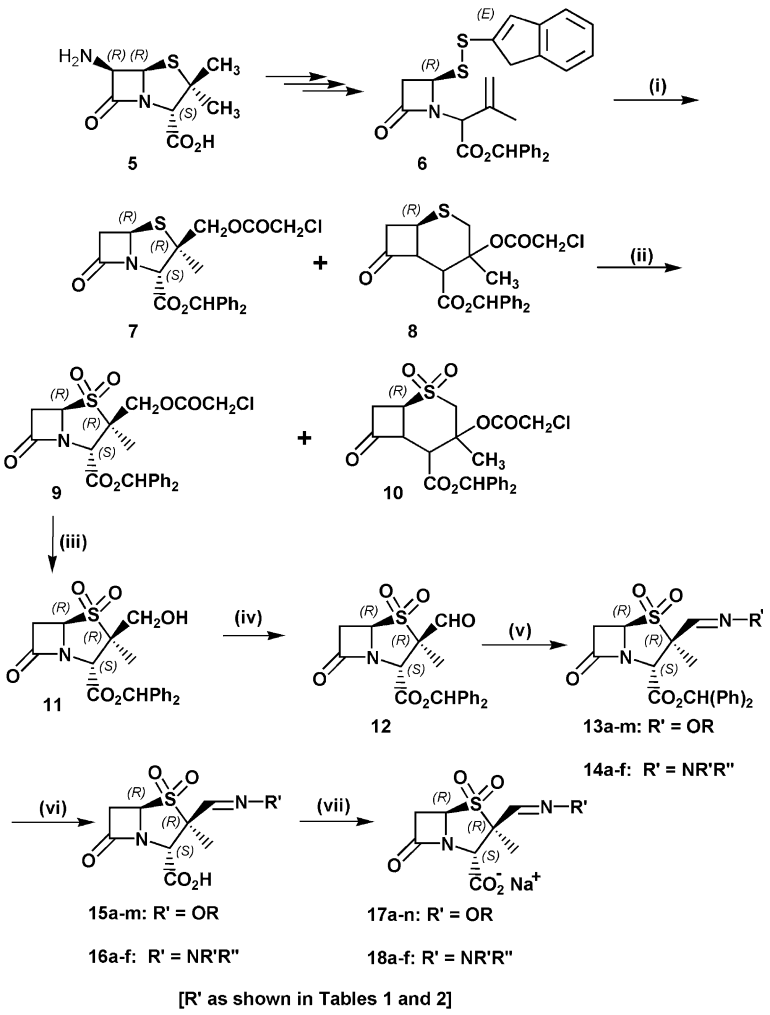
Deprotection of the carboxylic acid protecting group from the oxime and hydrazone series **13a**–**m** and **14a**–**f** (Table 1) under catalytic hydrogenation over 10% Pd/C/hydrogen at 50 psi in ethanol or by the use of trifluoroacetic acid gave the corresponding acids **15a**–**m** and **16a**–**f**, respectively (Scheme 1).

Treatment of the crude acids with aqueous $NaHCO_3$ solution followed by purification on a reverse-phase preparative TLC plates and lyophilization gave the corresponding sodium salts **17a**–**n** and **18a**–**f** (Table 2). Attempts to deprotect **13d** under catalytic hydrogenation over 10% P/C resulted in the simultaneous deprotection of the diphenylmethyl group and reduction of the allyl double bond to give the *O*-propyloxime derivative. Similarly, hydrogenation of the *O*-(pyridin-2-yl)methyloxime derivative **13m** over 10% P/C gave a mixture of the expected compound **17m** as a minor component in 7% yield and a major component *O*-(piperidin-2-yl)methyloxime derivative **17n** in 27% yield.

## 3. Results and discussion

The inhibitory activities of the new compounds against *E. coli* TEM-1, *Klebsiella pneumoniae* CTX-1 and *P. aeruginosa* cephalosporinase β-lactamases were evaluated in comparison with clinically useful inhibitors namely, clavulanic acid **1**, sulbactam **2a**, and tazobactam **2b**. The 50% inhibitory concentration ($IC_{50}$) values against these three β-lactamases are presented in Table 2. In the oxime series, the replacement of the hydroxyl moiety in **17a** with a methoxy **17b**, ethoxy **17c**, and allyloxy **17d** groups resulted in three to four times increased CTX-1 enzyme inhibitory activities from 0.02 to 0.006, 0.005, and 0.005 μM, respectively. Similarly, increases in activity were observed against the TEM-1 and cephalosporinase. In general, all the oxime derivatives **17a**–**n** strongly inhibited the TEM-1 enzyme, with **17i** and **17j** showing the lowest $IC_{50}$ of 0.007 and 0.0048 μM, respectively, compared with clavulanic acid (0.065 μM), sulbactam (4.5 μM), and tazobactam (0.065 μM). This strong level of β-lactamases inhibitory activity against the class A enzymes is typical of penam sulfones.[12,13]

All the oxime derivatives showed superior inhibitory activity against the TEM-1 enzyme in comparison to sulbactam, while the $IC_{50}$ values for clavulanic acid **1**




**Scheme 1.** Synthesis of 2β-substituted-oxyimino and -hydrazone penam sulfones. Reagents and conditions: (i) $ClCH_2CO_2H$, AgOAc; (ii) $KMnO_4$/AcOH/acetone; (iii) thiourea/ethanol; (iv) pyridinium chlorochromate, DCM; (v) R′$ONH_2$ or R′R″$NNH_2$/ethanol–DCM; (vi) TFA, anisole, 0 °C, $NaHCO_3$ or 10% Pd/C–ethanol, (vii) $NaHCO_3$ aqueous.

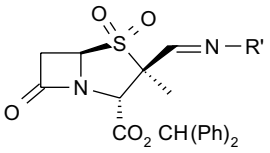
and tazobactam **2b** were about 14 times greater than that of **17j**. Unfortunately, this strong in vitro inhibitory activity did not translate into synergistic activity in combination with PIP; hence, compound **17j** and others with the exception of compounds **17g**, **17i**, and **17k** were devoid of synergistic activity against *E. coli* TEM-1 strain. Therefore, only compounds **17g**, **17i**, and **17k** enhanced the antibacterial activity of PIP against *E. coli* TEM-1 strain with MICs of 0.78, 0.39, and 0.78 μg/mL, respectively. The lack of synergy could be due to a variety of factors including poor uptake through the bacterial cell wall,[7] or the inhibitor could be overwhelmed by the hyperproduction of β-lactamases.[23,24] However, most of the compounds demonstrated synergistic activity comparable to that of tazobactam in combination with PIP against *E. coli* TEM-3 and TEM-7 strains (Table 3).

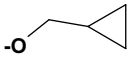
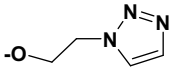
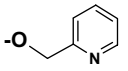
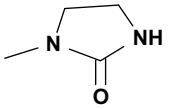
Furthermore, **17j** showed strong synergy with PIP against *E. coli* TEM-3, TEM-7, and OXA-1 strains by exhibiting >4-fold reduction in MIC values over PIP alone (Table 3). Similarly, in combination with CAZ, **17j** showed strong synergy against *E. coli* TEM-3, and TEM-7 with >4-fold reduction in MIC values compared with CAZ alone.

*K. pneumoniae* CTX-1 enzyme was strongly inhibited by all the oxyimino penam sulfone derivatives with the $IC_{50}$ values ranging from 0.0025 to 0.5 μM for **17j** and **17i**, respectively. In addition, both compounds **17j** and **17k** displayed the strongest inhibitory activity against the CTX-1 enzyme with $IC_{50}$ values of 0.0025 and 0.0028 μM, respectively. Removal of the *tert*-butyl protecting group of **17k** to give a more water soluble disodium salt **17j** did not significantly affect the β-lactamases inhibitory activity (Table 2). All the oxime derivatives demonstrated stronger synergistic activity against *K. pneumoniae* CTX-1 in combination with CAZ than with PIP. On other hand, TAZ did not protect CAZ against hydrolysis by the *K. pneumoniae* CTX-1 enzyme, despite the strong in vitro β-lactamase inhibitory activity demonstrated by TAZ, but only moderately protected PIP.

The *P. aeruginosa* cephalosporinase was only moderately inhibited by the oxime derivatives with $IC_{50}$ values

**Table 1.** Structures of 2β-substituted-oxyimino and -hydrazone penam sulfone esters



| Compounds | R′ |
|---|---|
| **13a** | –OH |
| **13b** | $-OCH_3$ |
| **13c** | $-OCH_2CH_3$ |
| **13d** | $-OCH_2CH{=}CH_2$ |
| **13e** | $-OCH_2CH_2OH$ |
| **13f** | $-OCH_2OCH_3$ |
| **13g** | $-OC(CH_3)_3$ |
| **13h** | $-OCH_2CONH_2$ |
| **13i** |  |
| **13j** | $-OCH_2CO_2CHPh_2$ |
| **13k** | $-OCH_2CO_2C(CH_3)_3$ |
| **13l** |  |
| **13m** |  |
| **14a** | –NHCOCH$_3$ |
| **14b** |  |
| **14c** | –NHCOPh |
| **14d** | $-NHCSNH_2$ |
| **14e** | $-NHCONH_2$ |
| **14f** | $-NHCOOCH_2Ph$ |

ranging from 0.45 to >10.0 μM for compounds **17k** and **17m**, respectively. A significant reduction in the cephalosporinase inhibition was observed by the substitution of the six-member heterocycles pyridine and piperidine as found in **17m** and **17n**, respectively. This moderate cephalosporinase inhibitory activity (Table 2) is a strong reflection of the observed weak to lack of synergistic activity in combination with either PIP or CAZ against cephalosporinase producing strains of *E. cloacae* P99 and *P. aeruginosa* (Tables 3 and 4). However, against *E. coli* strains producing other TEM (TEM-3 and TEM-7) and OXA-1 enzymes, the oxime series showed significant synergistic activity in combination with either PIP or CAZ.

In comparison to the oxime derivatives, the hydrazone penam sulfones were generally much weaker inhibitors of all the three β-lactamases. However, compound (**18a**, R′ = NHCOCH$_3$) showed the strongest TEM-1 enzyme inhibitory activity that is about seven and five times the activities of **17i** and **17j**, respectively. Although, compounds **18a**, **18b**, and **18c** did not show synergistic activity against *E. coli* TEM-1 in combination with PIP; they demonstrated strong synergy against *E. coli* TEM-3, TEM-7, and *S. aureus* strains. Against *E. coli* TEM-3, TEM-7, and *K. pneumoniae* CTX-1 these three compounds showed strong synergy in combination with CAZ. Furthermore, the hydrazone compounds **18a**, **18b**, and **18c** protected PIP against the *E. coli* TEM-3 and TEM-7 enzymes, but did not offer protection against *K. pneumoniae* CTX-1.

In conclusion, a series of 2β-oxyimino and -hydrazone penam sulfones have been synthesized and evaluated for their activity as β-lactamase inhibitors and as potential partners to protect β-lactamase labile β-lactam antibiotics. Most of the compounds had broad-spectrum inhibitory activity against the β-lactamases including the class A enzymes, normally sensitive to such compounds[10,19,25] and the class C enzymes; although activity against class C enzymes were markedly reduced compared to those against TEM-1 and CTX-1. This could be explained based on the understanding that high affinity with class C enzymes is dependent on C-6 substitution at the penam ring as noted for the penicillins. However, these 2β-substituted penam sulfones were stronger inhibitors of the serine β-lactamases, particularly the TEM-1 enzyme in comparison to Ro 48-1220 ($IC_{50}$, 1.1 μM).[17] The 2β-oxyimino penam sulfones demonstrated stronger inhibitory activity against all the three serine β-lactamases compared to the hydrazone derivatives. Overall, compounds **17b** (R′ = OCH$_3$) and **18a** (R′ = NHCOCH$_3$) were identified as the most active representative compounds from the oxyimino and hydrazone series, respectively. From a reversibility of β-lactamase inhibition study carried out on **17b** and **18a** it was show that very little enzyme activity was recovered after about 21 h of incubation of these compounds with TEM-1, CTX-1, and cephalosporinase enzymes (data not shown). This may be an indication that the 2β-substituted penam derivatives reported in this study will irreversibly inhibit these β-lactamases, with possibly a slow deacylation of the inhibited enzyme as reported for Ro 48-1220.

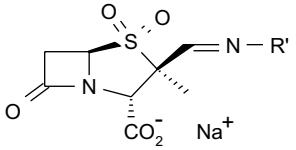
## 4. Experimental

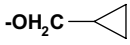
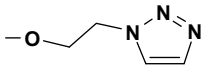
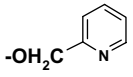
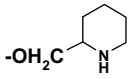
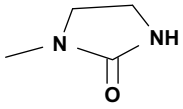
All column chromatographic purifications were accomplished on silica gel 60 (E. Merck, 230–400 mesh) using the appropriate solvent gradients. TLC was done on commercial reverse-phase silica gel plates (Analtech) containing calcium sulfate binder and fluorescent indicator. $^1$H NMR spectra were determined with a Bruker AC-200-F (200 MHz) spectrometer in appropriate deuterated solvents and are expressed in ppm downfield from TMS as internal standard. Elemental analyses were performed on a Carlo Erba Ea 11108 analyzer and results were within ±0.4% of theoretical values for C, H, and N.

### 4.1. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (12)[19]

To a stirred solution of diphenylmethyl (2*S*,3*R*,5*R*)-3-hydroxymethyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylate-4,4-dioxide[22] (**11**, 6.84 g, 16.46

**Table 2.** 50% Inhibitory concentration ($IC_{50}$, μM) values of penam sulfone derivatives



| Compounds | R′ | $IC_{50}$, (μM) values against | | |
|---|---|---|---|---|
| | | TEM-1 (*E. coli*) | CTX-1 (*K. pneumoniae*) | Cephalosporinase (*P. aeruginosa* 46012) |
| **17a** | –OH | 0.58 | 0.02 | 7.0 |
| **17b** | $-OCH_3$ | 0.3 | 0.006 | 1.9 |
| **17c** | $-OCH_2CH_3$ | 0.5 | 0.005 | 1.0 |
| **17d** | $-OCH_2CH{=}CH_2$ | 0.1 | 0.005 | 2.0 |
| **17e** | $-OCH_2CH_2OH$ | 0.5 | 0.007 | 1.4 |
| **17f** | $-OCH_2OCH_3$ | 0.1 | 0.0036 | 1.0 |
| **17g** | $-OC(CH_3)_3$ | 1.00 | 0.45 | 4.0 |
| **17h** | $-OCH_2CONH_2$ | 0.01 | 0.008 | 3.2 |
| **17i** |  | 0.007 | 0.5 | 1.4 |
| **17j** | $-OCH_2CO_2^-Na^+$ | 0.0048 | 0.0025 | 1.0 |
| **17k** | $-OCH_2CO_2C(CH_3)_3$ | 0.031 | 0.0028 | 0.45 |
| **17l** |  | 0.7 | 0.006 | 2.6 |
| **17m** |  | 0.08 | 0.01 | >10.0 |
| **17n** |  | 0.4 | 0.01 | 10 |
| **18a** | $-NHCOCH_3$ | 0.0014 | 0.1 | 0.6 |
| **18b** |  | n/t | n/t | n/t |
| **18c** | –NHCOPh | n/t | n/t | n/t |
| **18d** | $-NHCSNH_2$ | >10.0 | ~1.0 | >10.0 |
| **18e** | $-NHCONH_2$ | 2.5 | 0.1 | 1.0 |
| **18f** | $-NHCOOCH_2Ph$ | 3.0 | 0.1 | 1.0 |
| Clavulanic acid | | 0.065 | 0.0025 | >50 |
| Sulbactam | | 4.5 | 0.05 | 20 |
| Tazobactam (TAZ) | | 0.065 | 0.005 | 3 |

n/t = not tested.

mmol) in dry methylene chloride (340 mL), was added pyridinium chlorochromate (7.1 g) in one portion. The mixture was stirred at room temperature for 18 h, and an additional amount of pyridinium chlorochromate (1.78 g) was added, and the mixture was stirred for additional 5 h. The reaction mixture was diluted with methylene chloride, treated with charcoal, and filtered through a bed of Celite. The filtrate was washed with water, brine, dried ($Na_2SO_4$), and evaporated to give **12**, after flash column chromatography as a foam 5.14 g (76%). $^1$H NMR (DMSO-$d_6$): $\delta$ 9.85 (s, 1H), 7.23–7.38 (m, 10H), 6.85 (s, 1H), 5.82 (s, 1H), 5.25 (dd, 1H, $J$ = 1.2 Hz, $J$ = 4.43 Hz), 3.74 (dd, 1H, $J$ = 4.43 Hz, $J$ = 16.7 Hz), 3.30 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.7 Hz), 1.26 (s, 3H). Anal. ($C_{21}H_{19}NO_6S$) C, H, N.

### 4.2. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(hydroxyloxime)-4,4-dixoide (13a)

To a solution of intermediate aldehyde **12** (2.5 g, 6.05 mmol) in a mixture of methylene chloride (30 mL) and ethanol (60 mL) was added hydroxylamine hydrochloride (504 mg, 7.26 mmol) followed by pyridine (430 mg, 440 μL) and the mixture was stirred at room temperature for 5.5 h. The mixture was concentrated to dryness and the residue was taken up in ethyl acetate, washed with water, brine, dried ($Na_2SO_4$), and concentrated to give the crude product. Purification of the crude product over a silica gel column using benzene–ethyl acetate (10:1) gave 900 mg (35%) of **13a** as a foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 12.0 (s, 1H), 7.81 (s, 1H),

**Table 3.** Synergistic activity of penam sulfones **17a–m** and **18a–c** with PIP

| Test organisms | MIC (μg/mL) of | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PIP alone | +TAZ | **+17a** | **+17b** | **+17c** | **+17e** | **+17g** | **+17h** | **+17i** | **+17j** | **+17k** | **+17l** | **+17m** | **+18a** | **+18b** | **+18c** |
| *S. aureus* 54K | 12.5 | 0.78 | ⩽0.2 | 0.78 | 0.39 | 0.39 | 1.56 | 0.39 | ⩽0.2 | 0.39 | 0.78 | 0.39 | 1.56 | 0.39 | 0.78 | 0.39 |
| *S. aureus* 80K | 12.5 | 0.39 | ⩽0.2 | 0.78 | ⩽0.2 | ⩽0.2 | 0.78 | 0.78 | ⩽0.2 | 0.39 | ⩽0.2 | ⩽0.2 | 0.78 | ⩽0.2 | 0.78 | ⩽0.2 |
| *E. coli* TEM-1 | >50 | 0.39 | 12.5 | 6.25 | 12.5 | 25 | 0.78 | 50 | 0.39 | 50 | 0.78 | 25 | 12.5 | 25 | 50 | 25 |
| *E. coli* TEM-3 | >50 | 1.56 | 1.56 | 1.56 | 1.56 | 0.78 | 1.56 | 3.13 | 0.78 | 1.56 | 0.78 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 |
| *E. coli* TEM-7 | >50 | 0.78 | 6.25 | 0.78 | ⩽0.2 | ⩽0.2 | 0.39 | 6.25 | ⩽0.2 | 0.39 | 0.39 | 0.78 | 0.39 | 0.78 | 12.5 | 3.13 |
| *E. coli* OXA-1 | 25 | 3.13 | 6.25 | 0.78 | 1.56 | 1.56 | 0.78 | 6.25 | 1.56 | 6.25 | 0.78 | 6.25 | 1.56 | 12.5 | 25 | 25 |
| *K. pneumoniae* CTX-1 | >40 | 12.5 | 12.5 | 6.25 | 25 | 12.5 | 12.5 | >50 | 12.5 | 12.5 | 12.4 | >50 | 12.5 | 25 | 25 | >50 |
| *S. marcescens* 200L | >50 | 1.56 | 12.5 | <0.2 | 0.78 | 1.56 | 0.78 | 12.5 | 0.39 | 3.13 | 0.78 | 3.13 | 0.78 | 12.5 | 12.5 | 12.5 |
| *P. vulgaris* CT106 | >50 | 1.56 | >50 | 1.56 | 6.25 | 1.25 | 1.25 | >50 | 3.13 | 25 | 0.78 | 1.56 | 3.13 | >50 | >50 | >50 |
| *C. freundii* 2046E | >50 | 0.78 | 1.56 | 0.39 | 0.39 | 0.78 | ⩽0.2 | 6.25 | ⩽0.2 | 1.56 | ⩽0.2 | ⩽0.2 | ⩽0.2 | 3.13 | 12.5 | 25 |
| *E. cloacae* P99 | >50 | 50 | >50 | >50 | >50 | 25 | 50 | >50 | 50 | >50 | >50 | >50 | >50 | 12.5 | 25 | 50 |
| *P. aeruginosa* L. 46007 | >50 | 50 | 25 | 50 | 25 | 25 | 25 | 50 | 25 | 50 | 12.5 | 25 | 50 | 25 | 50 | 12.5 |
| *M. morganii* 36014 | 50 | <0.2 | 12.5 | ⩽0.2 | 0.39 | ⩽0.2 | ⩽0.2 | 6.25 | 0.39 | 3.13 | 0.78 | 25 | 1.56 | ⩽0.2 | 1.56 | 1.56 |

**Table 4.** Synergistic activity of penam sulfones **17a–m** and **18a–c** with CAZ

| Test organisms | MIC (μg/mL) of | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CAZ alone | +TAZ | **+17a** | **+17b** | **+17c** | **+17e** | **+17g** | **+17h** | **+17i** | **+17j** | **+17k** | **+17l** | **+17m** | **+18a** | **+18b** | **+18c** |
| *E. coli* TEM-3 | 25 | 0.39 | 0.78 | 0.39 | 0.39 | 0.39 | 0.39 | 3.13 | 0.39 | 0.78 | ⩽0.2 | 1.56 | 0.39 | 0.78 | 0.78 | 0.78 |
| *E. coli* TEM-7 | 25 | 12.9 | 12.5 | 0.39 | 0.39 | ⩽0.2 | 0.39 | 6.25 | 0.78 | 0.78 | 0.39 | 3.13 | ⩽0.2 | 1.56 | 3.13 | 3.13 |
| *K. pneumoniae* CTX-1 | >50 | >50 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 25 | 1.56 | 3.13 | 0.78 | 12.5 | 1.56 | 1.56 | 1.56 | 6.25 |
| *P. vulgaris* CT-106 | 12.5 | 12.5 | 3.13 | 0.39 | 0.78 | 0.78 | 0.78 | 12.5 | 0.39 | 1.56 | 0.39 | 0.39 | 1.56 | 6.25 | 12.5 | 6.25 |
| *E. cloacae* P99 | >50 | 12.5 | 50 | 12.5 | 25 | 12.5 | 25 | >50 | 25 | >50 | 12.5 | >50 | >50 | 6.25 | 6.25 | 6.25 |
| *P. aeruginosa* 46220 DR-2 | 25 | 1.56 | 25 | 12.5 | 25 | 12.5 | 6.25 | 12.5 | 12.5 | 12.5 | 25 | 12.5 | 12.5 | 12.5 | 25 | 12.5 |
| *E. aerogens* 41004 | 25 | 12.5 | 12.5 | 3.13 | 12.5 | 6.25 | 12.5 | 25 | 12.5 | 12.5 | 25 | 25 | 12.5 | 6.25 | 3.13 | 6.25 |
| *M. morganii* 36014 | 25 | <0.20 | 3.13 | ⩽0.2 | 0.78 | ⩽0.2 | ⩽0.2 | 6.25 | 3.13 | 3.13 | 0.78 | 25 | 1.56 | ⩽0.2 | 1.56 | 1.56 |

7.29–7.46 (m, 10H), 6.92 (s, 1H), 5.40 (s, 1H), 5.30 (br d, $J = 2.9$ Hz), 3.74 (dd, 1H, $J = 4.5$ Hz, $J = 16.6$ Hz), 3.32 (dd, 1H, $J = 1.2$ Hz, $J = 16.6$ Hz), 1.36 (s, 3H). Anal. ($C_{21}H_{20}N_2O_6S$) C, H, N.

### 4.3. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(hydroxyloxime)-4,4-dioxide (17a)

A mixture of compound **13a** (200 mg, 0.47 mmol) in ethanol (20 mL) and 10% Pd/C (200 mg) was hydrogenated at 50 psi for 5.5 h. The reaction mixture was filtered over a bed of Celite and the filtrate was concentrated under reduced pressure. The residue was triturated with a mixture of ether–hexane to give the crude as a white solid, which was collected by filtration (68 mg). The acid was dissolved in a solution of $NaHCO_3$ (24 mg) in water (10 mL), stirred at room temperature for 10 min, and freeze-dried to give a white solid (75 mg). The product was purified by reverse-phase preparative TLC using acetonitrile–water (7:1.5) as developing solvent, to give 30 mg (23%) of **17a** as white fluffy solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.56 (s, 1H), 7.42 (s, 1H), 5.00 (br d, 1H, $J = 2.9$ Hz), 4.27 (s, 1H), 3.54 (dd, 1H, $J = 4.6$ Hz, $J = 16.6$ Hz), 3.12 (dd, 1H, $J = 1.2$ Hz, $J = 16.5$ Hz), 1.53 (s, 3H). Anal. ($C_8H_9N_2NaO_6S$) C, H, N.

### 4.4. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-methyloxime)-4,4-dioxide (13b)

A suspension of the aldehyde **12** (1.0 g, 2.42 mmol) in 95% ethanol (50 mL) was treated with methoxylamine hydrochloride (242 mg, 2.903 mmol) followed by pyridine (230 mg, 2.903 mmol) and the mixture was stirred at room temperature for 5 h and concentrated under reduced pressure. The residue was dissolved in methylene chloride, washed with 5% HCl, water and brine. After drying ($Na_2SO_4$), the solvent was removed under reduced pressure to give 1.0 g of crude, which was purified over a silica gel column using hexane–ethyl acetate as eluent to give 467 mg (44%) of **13b** as a foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.89 (s, 1H); 7.22–7.40 (m, 10H), 6.86 (s, 1H), 5.39 (s, 1H), 5.26 (dd, 1H, $J = 1.4$ Hz, $J = 4.4$ Hz), 3.82 (s, 3H), 3.68 (dd, 1H, $J = 4.6$ Hz, $J = 16.7$ Hz), 3.26 (dd, 1H, $J = 1.4$ Hz, $J = 16.7$ Hz), 1.28 (s, 3H). Anal. ($C_{22}H_{22}N_2O_6S$) C, H, N.

### 4.5. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-methyloxime)-4,4-dioxide (17b)

To a solution of compound **13b** (294 mg, 0.664 mmol) in dry anisole (768 μL) at −5 °C under $N_2$, was added TFA (758 mg, 512 μL) and stirred at 0 °C for 3.5 h. The mixture was concentrated to dryness under reduced pressure and the residue was triturated with a mixture of ether–hexane, to the crude acid, which was collected by filtration (186 mg). The acid was treated with a solution of $NaHCO_3$ (113 mg) in water (10 mL), stirred at room temperature for 10 min, and freeze-dried to give a fluffy solid (229 mg), which was purified by reverse-phase preparative TLC using acetonitrile–water (7:1.5) as the developing solvent, to afford 44 mg (22%) of **17b** as a white fluffy solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.55 (s, 1H), 5.03 (br d, 1H, $J = 2.9$ Hz), 4.32 (s, 1H), 3.83 (s, 3H), 3.55 (dd, 1H, $J = 4.2$ Hz, $J = 16.1$ Hz), 3.12 (dd, 1H, $J = 1.3$ Hz, $J = 16.1$ Hz), 1.52 (s, 3H). Anal. ($C_9H_{11}N_2NaO_6S$) C, H, N.

### 4.6. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-ethyloxime)-4,4-dioxide (13c)

Compound **13c** was synthesized from intermediate **12** (1.83 g, 4.43 mmol) and ethyloxyamine hydrochloride (0.494 g, 4.43 mmol) as described for **13a**, to give 428 mg (23%) of **13c** as a foam after silica gel column purification using benzene–ethyl acetate (30:1). $^1$H NMR (DMSO-$d_6$): $\delta$ 7.93 (s, 1H), 7.25–7.50 (m, 10H), 6.92 (s, 1H), 5.45 (s, 1H), 5.32 (dd, 1H, $J = 1.2$ Hz, $J = 4.5$ Hz), 4.13 (q, 2H, $J = 7.0$ Hz), 3.75 (dd, 1H, $J = 4.5$ Hz, $J = 16.6$ Hz), 3.33 (dd, 1H, $J = 1.2$ Hz, $J = 16.6$ Hz), 1.36 (s, 3H), 1.21 (t, 3H, $J = 7.0$ Hz). Anal. ($C_{23}H_{24}N_2O_6S$) C, H, N.

### 4.7. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-ethyloxime)-4,4-dioxide (17c)

Compound **17c** was prepared from compound **13c** (0.298 g, 0.653 mmol) by hydrogenation over 10% Pd/C (300 mg) at 50 psi for 2.5 h as described for **17a**; and purified by reverse-phase preparative TLC plates to give 89 mg, (59%) of **17c** as a yellow fluffy solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.53 (s, 1H), 5.02 (br s, 1H), 4.34 (s, 1H), 4.09 (q, 2H, $J = 7.04$ Hz), 3.56 (dd, 1H, $J = 4.3$ Hz, $J = 16.1$ Hz), 3.13 (dd, 1H, $J = 1.1$ Hz, $J = 16.0$ Hz), 1.53 (s, 3H), 1.20 (t, 3H, $J = 7.04$ Hz). Anal. ($C_{10}H_{13}N_2NaO_6S$) C, H, N.

### 4.8. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-allyloxime)-4,4-dioxide (13d)

Compound **13d** was synthesized from the intermediate aldehyde **12** (2.23 g, 5.39 mmol) and allyloxyamine hydrochloride (709 mg, 6.47 mmol) as described for **13a** to give 800 mg (32%) of **13d** as a foam, after silica gel column purification using benzene–ethyl acetate (30:1). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.01 (s, 1H), 7.30–7.46 (m, 10H), 6.93 (s, 1H), 5.87–6.08 (m, 1H), 5.47 (s, 1H), 5.18–5.40 (m, 3H), 4.62 (d, 2H, $J = 5.6$ Hz), 3.76 (dd, 1H, $J = 4.5$ Hz, $J = 16.7$ Hz), 3.34 (dd, 1H, $J = 1.5$ Hz, $J = 16.7$ Hz), 1.35 (s, 3H). Anal. ($C_{24}H_{24}N_2O_6S$) C, H, N.

### 4.9. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-(*O*-allyloxime)-4,4-dioxide (17d)

Compound **17d** was prepared from **13d** (300 mg, 0.64 mmol) and anisole/TFA (768 μL/493 μL) as described for **17b** to give 15 mg (7%) of **17d** as a light

yellow fluffy solid after purification. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.59 (s, 1H), 5.90–6.05 (m, 1H), 5.20–5.40 (m, 2H), 5.02 (br d, 1H, $J$ = 2.9 Hz), 4.58 (d, 2H, $J$ = 5.6 Hz), 4.32 (s, 1H), 3.54 (dd, 1H, $J$ = 4.1 Hz, $J$ = 16.0 Hz), 3.14 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.0 Hz), 1.52 (s, 3H). Anal. ($C_{11}H_{13}N_2NaO_6S$) C, H, N.

### 4.10. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(2-hydroxyethyl)oxime]-4,4-dioxide (13e)

Compound **13e** was synthesized from intermediate **12** (1.5 g, 3.52 mmol) and 2-hydroxy-ethyloxyamine hydrochloride (0.955 g, 3.519 mmol) as described for **13a** to afford 202 mg (12%) of **13e** as a yellow gum, after silica gel column purification (benzene–ethyl acetate, 3:1). $^1$H NMR (DMSO-$d_6$): $\delta$ 7.95 (s, 1H), 7.29–7.45 (m, 10H), 6.93 (s, 1H), 5.46 (s, 1H), 5.31 (br d, 1H, $J$ = 2.5 Hz), 4.80 (t, 1H, $J$ = 5.4 Hz), 4.12 (t, 2H, $J$ = 5.0 Hz), 3.75 (dd, 1H, $J$ = 4.2 Hz, $J$ = 16.0 Hz), 3.60–3.72 (m, 2H), 3.35 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.0 Hz), 1.34 (s, 3H). Anal. ($C_{23}H_{24}N_2O_7S$) C, H, N.

### 4.11. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(2-hydroxyethyl)oxime]-4,4-dioxide (17e)

Compound **17e** was prepared from **13e** (188 mg, 0.398 mmol) by hydrogenation over 10% Pd/C (0.190 g) at 50 psi for 2.5 h, as described for **17a** to give 84 mg (58%) of **17e** as a pale yellow solid after purification. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.55 (s, 1H), 5.03 (br d, 1H, $J$ = 2.5 Hz), 4.78 (br s, 1H), 4.34 (s, 1H), 4.07 (t, 2H, $J$ = 4.9 Hz), 3.50–3.68 (m, 3H), 3.10 and 3.18 (2s, 1H), 1.53 (s, 3H). Anal. ($C_{10}H_{13}N_2NaO_7S$) C, H, N.

### 4.12. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(methoxymethyl)oxime]-4,4-dioxide (13f)

Compound **13f** was prepared in the same manner as described for compound **13b**; from intermediate **12** and methoxymethyloxyamine hydrochloride and purified by silica gel column chromatography to give 33% of **13f**. $^1$H NMR ($CDCl_3$): $\delta$ 7.64 (s, 1H), 7.28–7.40 (m, 10H), 6.93 (s, 1H), 5.17 (q, 2H, $J$ = 7.3 Hz), 5.09 (s, 1H), 4.64 (br t, 1H), 3.50 (t, 2H), 3.40 (s, 3H), 1.34 (s, 3H). Anal. ($C_{23}H_{24}N_2O_7S$) C, H, N.

### 4.13. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(methoxymethyl)oxime]-4,4-dioxide (17f)

Compound **17f** was prepared from **13f** (370 mg, 0.783 mmol) and 10% Pd/C (370 mg) at 50 psi for 5 h as described for **17a** after treating with a solution of $NaHCO_3$ (70 mg) in water, and purified to give 180 mg (70%) of **17f**. $^1$H NMR ($D_2O$): $\delta$ 7.81 (s, 1H), 5.17 (s, 2H), 5.09 (dd, 1H, $J$ = 1.6 Hz, $J$ = 4.2 Hz), 4.78 (s, 1H), 3.68 (dd, 1H, $J$ = 4.3 Hz, $J$ = 16.8 Hz), 3.48 (s, 3H), 3.40 (dd, 1H, $J$ = 1.6 Hz, $J$ = 16.8 Hz), 1.65 (s, 3H). Anal. ($C_{10}H_{13}N_2NaO_7S$) C, H, N.

### 4.14. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methy1-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(*O*-*t*-butyloxime)-4,4-dioxide (13g)

A solution of compound **12** (1.81 g, 4.39 mmol) in a mixture of ethanol (98%, 20 mL) and methylene chloride (15 mL) was treated with *t*-butoxylamine hydrochloride (0.5 g, 3.98 mmol) and triethylamine (0.282 g, 2.79 mmol). The mixture was stirred at room temperature overnight, and worked up as described for **13a**. Purification of the crude by silica gel column chromatography using hexane–ethyl acetate (3:2) gave 350 mg (16.5%) of **13g**. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.82 (s, 1H), 7.29–7.43 (m, 10H), 6.92 (s, 1H), 5.44 (s, 1H), 5.30 (dd, 1H, $J$ = 1.2 Hz, $J$ = 4.5 Hz), 3.74 (dd, 1H, $J$ = 4.5 Hz, $J$ = 16.6 Hz), 3.30 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.6 Hz), 1.38 (s, 3H), 1.24 (s, 9H). Anal. ($C_{25}H_{28}N_2O_6S$) C, H, N.

### 4.15. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(*O*-*t*-butyloxime)-4,4-dioxide (17g)

A solution of compound **13g** (315 mg, 0.65 mmol) in a mixture of ethyl acetate (6 mL) and ethanol (20 mL) was hydrogenated over 10% Pd/C (310 mg) at 50 psi for 8 h, and worked up as described for **17a**. The crude acid was treated with a solution of $NaHCO_3$ (55 mg) in water and freeze-dried to afford the crude product (200 mg), which was purified by reverse-phase preparative TLC (acetonitrile–water, 8:1) to yield 66 mg (30%) of **17g**. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.44 (s, 1H), 4.98 (dd, 1H, $J$ = 1.1 Hz, $J$ = 4.0 Hz), 4.33 (s, 1H), 3.53 (dd, 1H, $J$ = 4.3 Hz, $J$ = 16.0 Hz), 3.12 (dd, 1H, $J$ = 1.1 Hz, $J$ = 16.0 Hz), 1.54 (s, 3H), 1.25 (s, 9H). Anal. ($C_{12}H_{17}N_2NaO_6S$) C, H, N.

### 4.16. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(carboxamidomethyl)oxime]-4,4-dioxide (13h)

Compound **13h** was prepared from intermediate **12** (2.55 g, 6.163 mmol), carboxamidomethyloxy amine hydrochloride (0.65 g, 5.136 mmol), and pyridine (0.284 g), for 10 h as described for **13a**. Purification by silica gel column chromatography using hexane–ethyl acetate (1:1) gave 472 mg (16%) of **13h**. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.05 (s, 1H), 7.29–7.47 (m, 12H), 6.93 (s, 1H), 5.44 (s, 1H), 5.32 (d, 1H, $J$ = 3.0 Hz), 4.49 (s, 2H), 3.76 (dd, 1H, $J$ = 3.5 Hz, $J$ = 16.0 Hz), 3.34 (dd, 1H, $J$ = 1.1 Hz, $J$ = 16.0 Hz), 1.32 (s, 3H). Anal. ($C_{23}H_{23}N_3O_7S$) C, H, N.

### 4.17. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-(carboxamidomethyl)oxime]-4,4-dioxide (17h)

Compound **17h** was prepared from **13h** (380 mg, 0.783 mmol), 10% Pd/C (380 mg), and $NaHCO_3$ (66 mg) as described for **17g**, to give 190 mg (71%) of **17h** after purification. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.69 (s, 1H), 7.28 (d, 2H, $J$ = 19.6 Hz), 5.03 (br d, 1H), 4.43 (s, 2H), 4.31 (s, 1H), 3.55 (dd, 1H, $J$ = 4.0 Hz,

$J$ = 16.0 Hz), 3.14 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.51 (s, 3H). Anal. ($C_{10}H_{12}N_3NaO_7S$) C, H, N.

### 4.18. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(cyclopropylmethyl)oxime]-4,4-dioxide (13i)

Compound **13i** was prepared from intermediate **12** (2.17 g, 5.24 mmol), cyclopropyl-methyloxyamine hydrochloride (0.54 g, 4.37 mmol), and pyridine (283 μL) as described for **13a** to give **13i** 590 mg (23%) as a white foam after column purification. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.93 (s, 1H), 7.25–7.50 (m, 10H), 6.92 (s, 1H), 5.43 (s, 1H), 5.32 (dd, 1H, $J$ = 1.5 Hz, $J$ = 3.0 Hz), 3.92 (d, 2H, $J$ = 7.1 Hz), 3.75 (dd, 1H, $J$ = 4.5 Hz, $J$ = 16.6 Hz), 3.33 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.6 Hz), 1.36 (s, 3H), 1.00–1.20 (m, 1H), 0.44–0.55 (m, 2H), 0.23–0.30 (m, 2H). Anal. ($C_{25}H_{26}N_2O_6S$) C, H, N.

### 4.19. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(cyclopropylmethyl)oxime]-4,4-dioxide (17i)

Compound **17i** was prepared from compound **13i** (350 mg, 0.73 mmol), after hydrogenation over 10% Pd/C (450 mg) and treated with a solution of $NaHCO_3$ (61 mg) in water as described for **17g**, and purified to give 54 mg (21%) of **17i**. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.54 (s, 1H), 5.01 (br d, 1H, $J$ = 3.0 Hz), 4.30 (s, 1H), 3.90 (d, 2H, $J$ = 7.2 Hz), 3.53 (dd, 1H, $J$ = 4.2 Hz, $J$ = 16.2 Hz), 3.13 (dd, 1H, $J$ = 1.1 Hz, $J$ = 16.1 Hz), 1.52 (s, 3H), 1.03–1.20 (m, 1H), 0.47–0.57 (m, 2H), 0.22–0.30 (m, 2H). Anal. ($C_{12}H_{15}N_2NaO_6S$) C, H, N.

### 4.20. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(diphenylmethyloxycarbonylmethyl)oxime]-4,4-dioxide (13j)

To a solution of the aldehyde **12** (1.2 g, 2.90 mmol) in a mixture of ethanol (15 mL) and methylene chloride (15 mL), was added diphenylmethyloxycarbonyl methyloxyamine (2.90 mmol, generated in situ from diphenylmethyloxycarbonyl methyloxy phthalimido by treatment with hydrazine), and treated with two drops of concd HCl. The mixture was stirred for 14 h at room temp; and worked up as described for **13a**. Purification by column chromatography (hexane–ethyl acetate, 3:2), gave **13j** 645 mg (34%) as a foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.10 (s, 1H), 7.12–7.42 (m, 20H), 6.82 and 6.85 (2s, 2H), 5.46 (s, 1H), 5.30 (br d, 1H), 4.83 (ABq, 2H, $J$ = 16.0 Hz), 3.69 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.0 Hz), 3.25 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.25 (s, 3H). Anal. ($C_{36}H_{32}N_2O_8S$) C, H, N.

### 4.21. (2*S*,3*R*,5*R*)-3-Formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid-3[1]-[*O*-(carboxymethyl)oxime]disodium salt 4,4-dioxide (17j)

Compound **17j** was synthesized from compound **13j** (480 mg, 0.74 mmol), after hydrogenation over 10% Pd/C (500 mg) and treatment with a solution of $NaHCO_3$ (124 mg, 1.47 mmol) in water as described for **17g**, followed by usual purification and freeze-drying to give 33 mg (12%) of **17j**. $^1$H NMR ($D_2O$): $\delta$ 7.80 (s, 1H), 5.13 (dd, 1H, $J$ = 1.8 Hz, $J$ = 2.5 Hz), 4.82 (s, 1H), 4.59 (s, 2H), 3.73 (dd, 1H, $J$ = 4.3 Hz, $J$ = 16.8 Hz), 3.48 (dd, 1H, $J$ = 1.8 Hz, $J$ = 16.8 Hz), 1.68 (s, 3H). Anal. ($C_{10}H_{10}N_2Na_2O_8S$) C, H, N.

### 4.22. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(*t*-butyloxycarbonylmethyl)oxime]-4,4-dioxide (13k)

Compound **13k** was synthesized from the aldehyde **12** (326 mg, 0.79 mmol), *t*-butyloxycarbonylmethyloxyamine (116 mg, 0.79 mmol), and a drop of 6 M HCl, under similar conditions described for **13a**. Purified by silica gel column chromatography (hexane–ethyl acetate, 3:2) to give 131 mg (30.6%) of **13k**. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.08 (s, 1H), 7.29–7.48 (m, 10H), 6.92 (s, 1H), 5.48 (s, 1H), 5.33 (br d, 1H, $J$ = 3.0 Hz), 4.60 (ABq, 2H, $J$ = 12.0 Hz), 3.75 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.0 Hz), 3.34 (dd, 1H, $J$ = 1.1 Hz, $J$ = 16.0 Hz), 1.42 (s, 9H), 1.31 (s, 3H). Anal. ($C_{27}H_{30}N_2O_8S$) C, H, N.

### 4.23. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(*t*-butyloxycarbonylmethyl)oxime]-4,4-dioxide (17k)

Compound **17k** was obtained from **13k** (317 mg, 0.584 mmol) after hydrogenation over 10% Pd/C (300 mg) as described for **17g**. Treatment with a solution of $NaHCO_3$ (26 mg) in water, and purification gave **17k** 45 mg (36%). $^1$H NMR (DMSO-$d_6$): $\delta$ 7.66 (s, 1H), 5.02 (br d, 1H, $J$ = 2.9 Hz), 4.55 (br d, 2H), 4.34 (s, 1H), 3.55 (dd, 1H, $J$ = 4.1 Hz, $J$ = 16.0 Hz), 3.15 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.51 (s, 3H), 1.43 (s, 9H). Anal. ($C_{14}H_{19}N_2NaO_8S$) C, H, N.

### 4.24. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-[2-(1*H*-1,2,3-triazol-1-yl)ethyl]oxime]-4,4-dioxide (13l)

Compound **13l** was synthesized from the penam aldehyde **12** (3.3 g, 7.98 mmol), 1,2,3-triazol-1-ylethyloxyamine hydrochloride (0.87 g, 7.98 mmol) and pyridine (0.5 g) as described for **13a**, followed by purification by silica gel column chromatography (hexane–ethyl acetate) to give 900 mg (22%) of **13l** as foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.98 and 8.00 (2s, 2H), 7.70 (s, 1H), 7.33–7.40 (m, 10H), 6.92 (s, 1H), 5.46 (s, 1H), 5.33 (br d, 1H, $J$ = 3.0 Hz), 4.65 (br d, 2H), 4.47 (br t, 2H), 3.75 (dd, 1H, $J$ = 4.0 Hz, $J$ = 15.1 Hz), 3.32 (dd, 1H, $J$ = 1.0 Hz, $J$ = 15.0 Hz), 1.33 (s, 3H). Anal. ($C_{25}H_{25}N_5O_6S$) C, H, N.

### 4.25. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3[1]-[*O*-(2-(1*H*-1,2,3-triazol-1-yl)ethyl)oxime]-4,4-dioxide (17l)

Compound **17l** was prepared from compound **13l** (400 mg, 0.764 mmol) after hydrogenation over 10% Pd/C (420 mg) as described for **17h**, and purified to give

170 mg (59%) of **17l** as a fluffy solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.16 (s, 1H), 7.70 (s, 1H), 7.58 (s, 1H), 5.05 (br d, 1H), 4.60–4.72 (m, 2H), 4.38–4.49 (m, 2H), 4.35 (s, 1H), 3.54 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.0 Hz), 3.14 (dd, 1H, $J$ = 0.9 Hz, $J$ = 16.0 Hz), 1.55 (s, 3H). Anal. ($C_{12}H_{14}N_5NaO_6S$) C, H, N.

### 4.26. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-[(pyridin-2-yl)methyl]oxime]-4,4-dioxide (13m)

Compound **13m** was prepared from the penam aldehyde **12** (3.5 g, 8.47 mmol), pyridin-2-ylmethyloxyamine hydrochloride (1.23 g) and pyridine (0.964 g) as described for **13a**, and purified by silica gel column chromatography (hexane–ethyl acetate) to give 700 mg (16%) of **13m**. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.54–8.57 (m, 1H), 8.13 (s, 1H), 7.66–7.74 (m, 1H), 7.30–7.45 (m, 12H), 6.91 (s, 1H), 5.49 (s, 1H), 5.32 (dd, 1H, $J$ = 1.0 Hz, $J$ = 3.0 Hz), 5.24 (s, 2H), 3.75 (dd, 1H, $J$ = 4.5 Hz, $J$ = 16.6 Hz), 3.34 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.6 Hz), 1.31 (s, 3H). Anal. ($C_{27}H_{25}N_3O_6S$) C, H, N.

### 4.27. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-[(pyridin-2-yl)methyl]oxime]-4,4-dioxide (17m)

This compound was prepared from **13m** (410 mg, 0.789 mmol) hydrogenated over 10% Pd/C (410 mg) as described for **17l**, and purified to give 20 mg (7%) of **17m** as the minor component. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.56 (d, 1H), 7.78–7.86 (m, 1H), 7.72 (s, 1H), 7.34–7.43 (m, 2H), 5.20 (s, 2H), 5.04 (br d, 1H), 4.36 (s, 1H), 3.56 (dd, 1H, $J$ = 4.0 Hz, $J$ = 16.0 Hz), 3.15 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.1 Hz), 1.52 (s, 3H). Anal. ($C_{14}H_{14}N_3NaO_6S$) C, H, N.

### 4.28. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-[*O*-[(piperidin-2-yl)methyl]oxime]-4,4-dioxide (17n)

Compound **17n** was obtained as the major component as a fluffy solid 80 mg (27%). $^1$H NMR ($D_2O$): $\delta$ 7.76 (d, 1H, $J$ = 2.2 Hz), 5.11 (br d, 1H), 4.79 (s, 1H), 4.18–4.45 (m, 2H), 3.49 (dd, 1H, $J$ = 4.2 Hz, $J$ = 16.8 Hz), 3.38–3.61 (br m, overlapped with a d, 3H, $J$ = 16.8 Hz), 2.99 (t, 1H, $J$ = 9.0 Hz), 1.46–1.89 (m, overlapped with a s, 9H). Anal. ($C_{14}H_{20}N_3NaO_6S$) C, H, N.

### 4.29. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(acetylhydrazone)-4,4-dioxide (14a)

To a stirred solution of penam aldehyde **12** (1.2 g, 2.9 mmol) in a mixture of ethanol (15 mL) and methylene chloride (15 mL), was added acetic hydrazide (221 mg, 2.9 mmol) followed by one drop of concd HCl. The mixture was stirred at room temperature for 14 h; the solvent was removed under reduced pressure and the residue was taken up in methylene chloride (100 mL), washed with water, brine, and dried ($Na_2SO_4$). Purification by silica gel column chromatography (hexane–ethyl acetate) gave 400 mg (29%) of **14a**. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.69 (s, 1H, exchanged with $D_2O$), 7.56 (s, 1H), 7.30–7.50 (m, 10H), 6.90 (s, 1H), 5.77 (s, 1H), 5.34 (br d, 1H), 3.75 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.1 Hz), 3.50 (dd, 1H, $J$ = 1.2 Hz, $J$ = 16.0 Hz), 2.00 (s, 3H), 1.39 (s, 3H). Anal. ($C_{23}H_{23}N_3O_6S$) C, H, N.

### 4.30. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(acetylhydrazone)-4,4-dioxide (18a)

A solution of the **14a** (320 mg, 0.68 mmol) in a mixture of ethanol (12 mL) and ethyl acetate (18 mL), was hydrogenated over 10% Pd/C (350 mg) at 50 psi for 8 h; and worked up as described for **17a** to give the crude acid. The crude acid was treated with a solution $NaHCO_3$ (57 mg) in water (10 mL) and the solution was freeze-dried to give a solid, which was purified by reverse-phase preparative TLC (acetonitrile–water, 7:1), to give 50 mg (23%) of **18a**. $^1$H NMR ($D_2O$): $\delta$ 7.69 (s, 1H), 5.13 (br d, 1H), 4.88 (s, 1H), 3.73 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.0 Hz), 3.40 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 2.12 (s, 3H), 1.71 (s, 3H). Anal. ($C_{10}H_{12}N_3NaO_6S$) C, H, N.

### 4.31. Diphenylmethyl (2*S*,3*R*,5*R*)-3-methyl-7-oxo-3-[*N*-(2-oxo-1-imidazolidinyl)formimidoyl]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (14b)

Compound **14b** was prepared from the aldehyde **12** (600 mg, 1.21 mmol) and 1-amino-2-oxo-imidazolidine (122 mg, 1.21 mmol) as described for **14a**. Purification by silica gel column chromatography (hexane–ethyl acetate, 4:1) gave 190 mg (26%) of **14b** as a foam. $^1$H NMR ($CDCl_3$): $\delta$ 7.23–7.40 (m, 10H), 6.95 (s, 1H), 6.89 (s, 1H), 5.40–5.62 (br m, 1H, exchanged with $D_2O$), 5.05 (s, 1H), 4.69 (br t, 1H), 3.55–3.80 (m, 4H), 3.42–3.55 (m, 2H), 1.52 (s, 3H). Anal. ($C_{24}H_{24}N_4O_6S$) C, H, N.

### 4.32. Sodium (2*S*,3*R*,5*R*)-3-methyl-7-oxo-3-[*N*-(2-oxo-1-imidazolidinyl)formimidoyl]-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylate-4,4-dioxide (18b)

A solution of **14b** (190 mg, 0.383 mmol) in ethanol (20 mL) was hydrogenated over 10% Pd/C (200 mg) at 50 psi for 2.5 h; and worked up as described for **17a**. The crude acid was treated with a solution of $NaHCO_3$ (40 mg) in water (10 mL), and freeze-dried to give a solid, which was purified by reverse-phase preparative TLC to give 17 mg (12%) of **18b**. $^1$H NMR ($D_2O$): $\delta$ 6.97 (s, 1H), 5.10 (dd, 1H, $J$ = 1.6 Hz, $J$ = 4.0 Hz), 4.77 (s, 1H), 3.50–3.88 (m, 5H), 3.40 (dd, 1H, $J$ = 1.6 Hz, $J$ = 16.6 Hz), 1.69 (s, 3H). Anal. ($C_{11}H_{13}N_4NaO_6S$) C, H, N.

### 4.33. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicylco[3.2.0]heptane-2-carboxylate-3$^1$-(benzoylhydrazone)-4,4-dioxide (14c)

Compound **14c** was prepared from the aldehyde **12** (0.8 g, 1.94 mmol) and benzoic hydrazide (0.263 g,

1.94 mmol) as described for **14a**. Purification by silica gel column chromatography (hexane–ethyl acetate) gave 530 mg (51%) of **14c** as a foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.07 (s, 1H), 7.28–8.04 (m, 16H), 6.88 (s, 1H), 5.40 (br s, 1H), 5.31 (s, 1H), 3.79 (dd, 1H, $J$ = 4.0 Hz, $J$ = 16.0 Hz), 3.36 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.47 (s, 3H). Anal. ($C_{28}H_{25}N_3O_6S$) C, H, N.

### 4.34. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(benzoylhydrazone)-4,4-dioxide (18c)

Compound **18c** was prepared from the ester **14c** (310 mg, 0.58 mmol), hydrogenated over 10% Pd/C (350 mg) as described for **18a**, treated with a solution of $NaHCO_3$ (41 mg) in water, and purified on reverse-phase preparative TLC to give 100 mg (59%) of **18c**. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.80–8.05 (m, 3H), 7.40–7.60 (m, 4H), 5.07 (br s, 1H), 4.30 (br s, 1H), 3.58 (dd, 1H, $J$ = 3.0 Hz, $J$ = 16.0 Hz), 3.17 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.1 Hz), 1.19 (s, 3H). Anal. ($C_{15}H_{14}N_3NaO_6S$) C, H, N.

### 4.35. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-thiosemicarbazone-4,4-dioxide (14d)

Compound **14d** was prepared from the penam aldehyde **12** (1.7 g, 4.11 mmol) and thiosemicarbazide (0.375 g, 4.11 mmol), as described for **14a** and purified by silica gel column chromatography (hexane–ethyl acetate) to give 900 mg (45%) of **14d**. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.75 (br s, 1H, exchanged with $D_2O$), 8.41 (br s, 1H, exchanged with $D_2O$), 8.06 (br s, 1H, exchanged with $D_2O$), 7.46 (s, 1H), 7.20–7.40 (m, 10H), 6.80 (s, 1H), 5.45 (s, 1H), 5.25 (br d, 1H, $J$ = 2.5 Hz), 3.65 (dd, 1H, $J$ = 4.4 Hz, $J$ = 16.1 Hz), 3.22 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.35 (s, 3H). Anal. ($C_{22}H_{22}N_4O_5S_2$) C, H, N, S.

### 4.36. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-thiosemicarbazone-4,4-dioxide (18d)

Compound **18d** was prepared from **14d** (300 mg, 0.617 mmol) and 10% Pd/C (600 mg) and $H_2$, as described for **18a**. Treatment with a solution of $NaHCO_3$ (47 mg), and purification on reverse-phase preparative TLC gave 38 mg (18%) of **18d**. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.05 (br s, 1H), 7.93 (br s, 1H), 7.61 (s, 1H), 7.44 (br s, 1H), 6.51 (s, 1H), 4.20 (br d, 1H), 3.10 (br d, 1H, $J$ = 14.9 Hz), 2.80 (dd, 1H, $J$ = 3.2 Hz, $J$ = 15.0 Hz), 1.90 (s, 3H). Anal. ($C_9H_{11}N_4NaO_5S_2$) C, H, N.

### 4.37. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-semicarbazone-4,4-dioxide (14e)

To a stirred solution of the penam aldehyde **12** (2.3 g, 5.6 mmol) in a mixture of ethanol (10 mL) and methylene chloride (10 mL) was treated with semicarbazide hydrochloride (0.477 g, 4.3 mmol) and pyridine (0.204 g, 2.58 mmol). The reaction was worked up as described for **14a** to give the crude foam (2.4 g). Purification by silica gel column chromatography (hexane–ethyl acetate, 1:1) gave 480 mg (18%) of **14e**. $^1$H NMR (DMSO-$d_6$): $\delta$ 10.67 (s, 1H), 7.25–7.48 (m, 10H), 6.92 (s, 1H), 6.61 (br s, 2H), 5.39 (s, 1H), 5.33 (br d, 1H, $J$ = 2.90 Hz), 3.73 (dd, 1H, $J$ = 4.5 Hz, $J$ = 16.5 Hz), 3.34 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.5 Hz), 1.43 (s, 3H). Anal. ($C_{22}H_{22}N_4O_6S$) C, H, N, S.

### 4.38. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-semicarbazone-4,4-dioxide (18e)

This compound was prepared as described for compound **18a**, from compound **14e** (380 mg, 0.81 mmol), by hydrogenation over 10% Pd/C at 50 psi for 24 h and treatment with a solution of $NaHCO_3$ (68 mg) in water. Purification on reverse-phase preparative TLC gave **18e** as a fluffy solid 126 mg (62%). $^1$H NMR (DMSO-$d_6$): $\delta$ 10.42 (br s, 1H), 7.22 (s, 1H), 6.33 (br s, H), 4.97 (br s, 1H), 4.19 (s, 1H), 3.53 (dd, 1H, $J$ = 4.0 Hz, $J$ = 16.0 Hz), 3.10 (dd, 1H, $J$ = 1.0 Hz, $J$ = 15.9 Hz), 1.52 (s, 3H). Anal. ($C_9H_{11}N_4NaO_6S$) C, H, N.

### 4.39. Diphenylmethyl (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(benzyloxycarbonyl-hydrazone)-4,4-dioxide (14f)

Compound **14f** was prepared from the penam aldehyde **12** (2.0 g, 4.84 mmol), *N*-benzyloxycarbonyl hydrazine hydrochloride (891 mg, 4.3978 mmol), and pyridine (313 mg, 320 μL) as described for **14e**. Purification by silica gel column chromatography (hexane–ethyl acetate) gave 745 mg (27%) of **14f** as a foam. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.75 (br s, 1H), 7.61 (s, 1H), 7.22–7.41 (m, 15H), 6.87 (s, 1H), 5.20–5.40 (m, 4H), 3.75 (dd, 1H, $J$ = 4.7 Hz, $J$ = 16.6 Hz), 3.32 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.6 Hz), 1.38 (s, 3H). Anal. ($C_{29}H_{27}N_3O_7S$) C, H, N, S.

### 4.40. Sodium (2*S*,3*R*,5*R*)-3-formyl-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-3$^1$-(benzyloxycarbonyl-hydrazone)-4,4-dioxide (18f)

Compound **18f** was prepared by catalytic hydrogenation of **14f** (292 mg, 0.520 mmol), over 10% Pd/C at 50 psi for 24 h as described for **18a**. Treatment with a solution of $NaHCO_3$ in water, followed by purification on reverse-phase TLC gave 26 mg (12%) of **18f** as a white fluffy solid. $^1$H NMR (DMSO-$d_6$): $\delta$ 11.34 (br s, 1H), 7.23–7.45 (m, 5H), 5.11 (s, 2H), 4.97 (br d, 1H, $J$ = 2.9 Hz), 4.15 (s, 1H), 3.52 (dd, 1H, $J$ = 3.9 Hz, $J$ = 16.0 Hz), 3.10 (dd, 1H, $J$ = 1.0 Hz, $J$ = 16.0 Hz), 1.50 (s, 3H). Anal. ($C_{16}H_{16}N_3NaO_7S$) C, H, N.

## 5. Assay of β-lactamase activity and β-lactamase inhibitory activity

The β-lactamase enzymes were obtained as crude cell extracts (from *E. coli* TEM-1, *K. pneumoniae* CTX-1, and *P. aeruginosa* 46012 cephalosporinase) prepared by

ultrasonication. β-Lactamase activity was determined by the UV method.[26] The amount of protein was measured by the Bio-Rad Protein Assay. The β-lactamase inhibitory activity was performed by UV spectrophotometry using freshly prepared antibiotic solutions in 50 mM phosphate buffer at pH 7.4. The assay was run at 30 °C, by monitoring the hydrolysis of the substrates at the following wavelengths; 235 nm for ampicillin (100 μM) and 260 nm for cephaloridine (100 μM) as substrates. The substrates were added to the reaction mixture after pre-incubation of the enzyme with β-lactamase inhibitor for 5 min. Clavulanic acid, sulbactam and tazobactam were used as reference drugs in the determination of 50% inhibitory concentration ($IC_{50}$, μM) values.

## 6. In vitro susceptibility testing

The in vitro synergistic antibacterial activities were determined by the agar dilution method. A total of $10^6$ cells of bacterial suspension per mL were spotted on the Mueller Hinton agar containing the antibiotics (piperacillin, PIP or ceftazidime, CAZ) alone or in combination with 5 μg/mL of the inhibitor. The minimum inhibitory concentration (MIC, μg/mL) values defined as the lowest antibiotic concentration that prevented visible bacterial growth were recorded after incubation overnight at 37 °C. Tazobactam was synthesized in our laboratories, while ceftazidime, clavulanic acid, piperacillin, and sulbactam were obtained as commercial preparations.

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## References and notes

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