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Azetidinone-isothiazolidinones: Stereoselective synthesis and antibacterial evaluation of new monocyclic beta-lactams

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ABSTRACT

A simple and efficient procedure for the stereoselective synthesis of new azetidinone-isothiazolidinones has been developed. New compounds were tested in vitro on a panel of Gram-positive and Gram-negative bacterial pathogens, some of them showing weak antibacterial activity.

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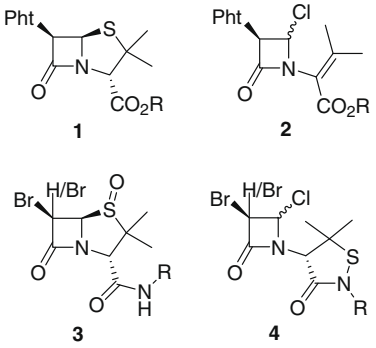
1. Introduction

Beta-lactam antibiotics are still the main drugs to treat infections caused by bacteria. These molecules disturb the final step of bacterial cell wall biosynthesis by inhibiting penicillin binding proteins (PBPs) involved in the crosslinking of peptidoglycan strands.[1]

The first classes of monocyclic beta-lactam antibacterial agents were isolated from natural sources in the late 1970s and early 1980s. The discovery of nocardicines and monobactams (Fig. 1) demonstrated for the first time that a conformationally constrained bicyclic structure was not necessary for antibacterial activity.[2–5] Research efforts in this class led to preparation of semi-synthetic and synthetic monobactam analogues like Aztreonam and Carumonam (Fig. 1), active mostly against Gram-negative bacteria. Work in this area is still very active and new compounds are synthesized and reported as inhibitors of D,D-transpeptidase,[6] beta-lactamase,[7] mammalian elastase,[8,9] fatty acid amide hydrolase[10,11] and cholesterol uptake.[12] Some of the compounds out of this class have also shown antifungal[13] and antitubercular activity.[14]

One of commonly used methods for the preparation of monocyclic beta-lactams from natural penicillins is an electrophilic ring opening of thiazolidine moiety by chlorinating agents.[15–19] In the case of phthalimido-penam esters **1**, a mixture of *cis*- and *trans*-chloro-azetidinone derivatives **2** was formed.[15] The ring opening of sulfoxide derivatives **3** and rearrangement to isothiazolidinone-azetidinones **4** by thionyl chloride was mentioned in the patent application.[20]



Azetidinone-isothiazolidinones, a new class of monocyclic beta-lactams, represent very interesting structures with two uncondensed heterocyclic rings that could both act as a potential pharmacophore. As a part of our work on the transformation of natural penicillins into new monocyclic beta-lactam derivatives[21,22] with potential biological activity, the synthesis of new azetidinone-isothiazolidinones was investigated. In this paper we report a simple and efficient procedure for the preparation of

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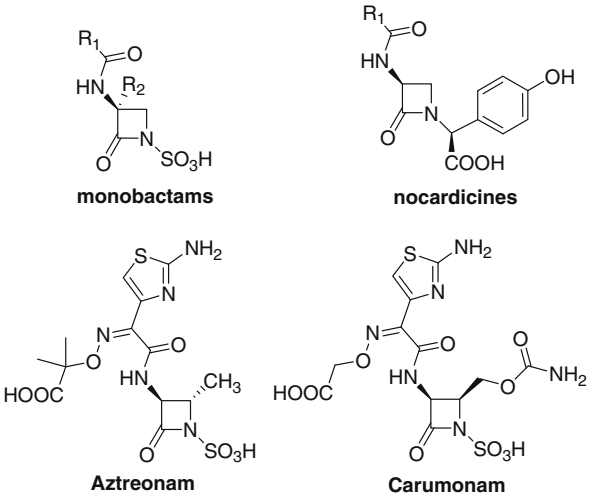


Figure 1. Natural and synthetic monocyclic beta-lactams.

new phthalimido-azetidinone-thiazolidinones **10–17**, starting from new penam amides, and evaluation of their antibacterial activity.

2. Results and discussion

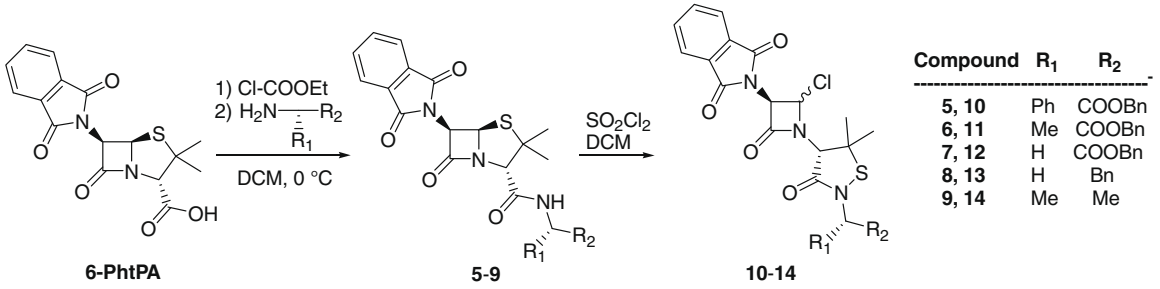
2.1. Chemistry

Preparation of new compounds **5–14** is depicted on Scheme 1. Reaction course and formation of products was monitored by LC/MS. 6-Phthalimido-penam amides **5–9** were prepared in good yields (76–94%) by amidation of previously synthesized[23] 6-phthalimido-penicillanic acid (6-PhtPA). Amidation was carried out by a method commonly applied in organic synthesis and in beta-lactam chemistry.[24] The reaction of corresponding phthalimido-penam amides **5–9** with sulfuryl chloride gave azetidinone-isothiazolidinones **10–14** as a mixture of *cis*- and *trans*-diastereomers. The obtained diastereomeric pairs **10–14** were separated by column chromatography. The use of thionyl chloride for the ring opening of phthalimido-penam amides, as mentioned in the literature,[20] was not successful. The reaction did not proceed at room temperature, while under heating a multicomponent complex mixture of undesired products was obtained.

Thiazolidine ring opening of penam amides **5–9** is regiospecific and highly stereoselective (Scheme 2). Monitoring the reaction course by LC/MS the formation of two intermediates was observed, presumably *cis*- and *trans*-**A**, which undergo intramolecular cyclization to *cis*- and *trans*-azetidinone-isothiazolidinones **10–14**, respectively. The ratio of the diastereoisomers, determined by LC/MS, clearly shows the predominance of the *trans*-isomers which could be explained by steric hindrance of the bulky phthalimide (Pht) substituent on beta-lactam ring.

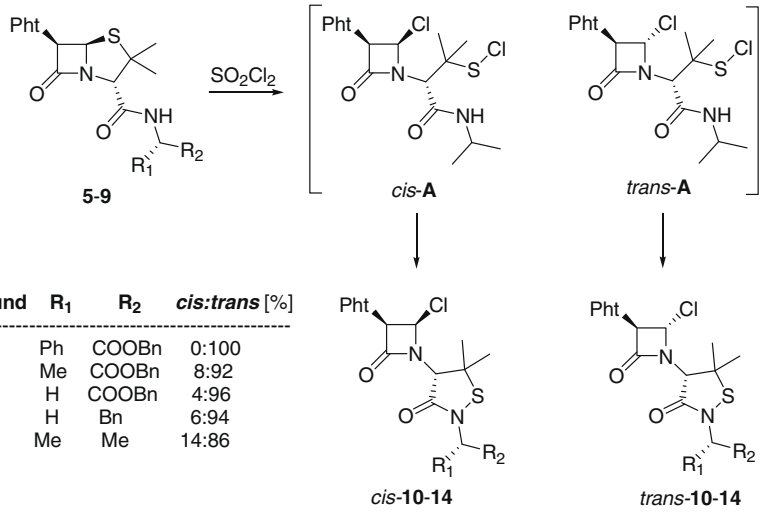
Benzyl esters of compounds *trans*-**10–12** were transformed to carboxylic acid derivatives *trans*-**15–17** (Scheme 3) by treatment with aluminium chloride in nitromethane.

All new compounds **5–17** were fully characterized by spectroscopic and analytical data. Amide derivatives **5–9** in ^{1}H NMR spectra show characteristic NH signals in the range of 8.1–9.2 ppm with corresponding splitting pattern, which disappear after a D_2O shake. The structure of compounds **10–14** was evident from C_2–H chemical shifts to lower field, due to introduction of chlorine at that



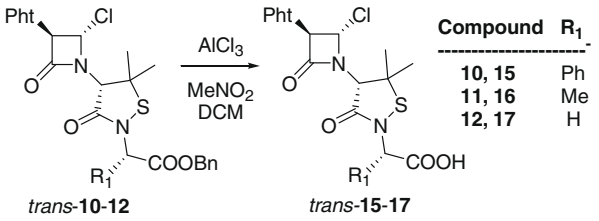
Compound	R_1	R_2
5, 10	Ph	COOBn
6, 11	Me	COOBn
7, 12	H	COOBn
8, 13	H	Bn
9, 14	Me	Me

Scheme 1. Synthesis of new compounds **5**–**14**.



Compound	R_1	R_2	*cis:trans* [%]
10	Ph	COOBn	0:100
11	Me	COOBn	8:92
12	H	COOBn	4:96
13	H	Bn	6:94
14	Me	Me	14:86

Scheme 2.



Scheme 3. Synthesis of new azetidinone-isothiazolidinones *trans*-**15**–**17**.

position and thiazolidine ring opening. Isothiazolidinone ring closure by S–N bond formation was evident from the absence of NH signals, present in amides **5–9**. The configuration of compounds *trans*-**10–14** and *trans*-**15–17** is based on the beta-lactam coupling constants of 1.3–1.8 Hz, characteristic for *trans*-configuration. In compounds **5–9** and *cis*-**11–14** the coupling constants are 3.9–4.3 Hz, in accordance with the *cis*-configuration of the beta-lactam ring.

2.2. In vitro screen

New compounds **5–17** were tested in vitro on a panel of selected Gram-positive (*Staphylococcus aureus, Enterococcus faecalis* and *Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli, Haemophilus influenzae* and *Moraxella catarrhalis*) bacterial strains (Table 1). The selected set of strains represents well described reference strains obtained from ATCC bioresource center.

The activity of penicillin amides **5–9** against selected set of bacterial strains could not be precisely determined since the MICs were generally higher than the standard cut-off value (128 μg/mL) used in our in vitro screen. These compounds could be considered as antibacterially inactive.

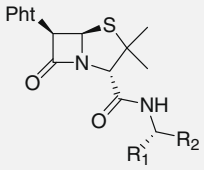
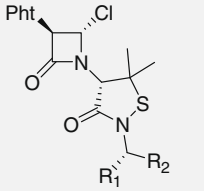
Several compounds from the azetidinone-isothiazolidinone class showed some improved antibacterial activity compared to penicillin amides. The activity of this class of compounds against Gram-positive strains was somewhat superior when compared to Gram-negative strains. It is evident that aromatic substituents R_1 and R_2 on isothiazolidinone nitrogen favour antibacterial activity. Compounds **14–17** that have no aromatic substituents on isothiazolidinone nitrogen were generally inactive against all tested strains, while some modest activity could be observed with compounds **10–13**, irrespective of their diastereomeric form. In line with that, the removal of aromatic benzyl ester group in compounds *trans*-**10–12** into carboxylic acids *trans*-**15–17** resulted in loss of antibacterial activity. Compound **10**, with two aromatic R_1 and R_2 substituents, showed the best inhibitory activity against selected Gram-positive strains with the best growth inhibition of *S. pneumoniae* (MIC 8 μg/mL). Similar SAR was observed with some previously described 4-amino-isothiazolidinone derivatives.[25] These compounds were found to be chemically instable, that could potentially contribute to the poor antibacterial activity.

3. Experimental

3.1. General procedures

All melting points were determined on Büchi B-540 apparatus in capillary tubes and are uncorrected. IR spectra were recorded on KBr discs on a Perkin–Elmer SpectrumGX FT-IR spectrophotometer with a resolution of 4 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance 300 and Bruker Avance 600 spectrometers with TMS as an internal standard. Column chromatography was carried out on silica gel Merck Kieselgel 60, 0.04–0.063 mm. HPLC was performed on Agilent 1100 instrument with DAD detector and ESI LC/MSD trap on column Phenomenex Gemini C18 with gradient elution (acetonitrile/ammonium acetate buffer, pH 7.4 = 20:80 to 90:10). High Resolution Mass Spectra (HRMS) were recorded on Micromass Q-Tof micro™ instrument equipped with the lock-spray (Waters Corporation, Manchester, UK). Elemental analysis was carried out on Perkin–Elmer 2400 Series II CHNS/O analyzer. Water content was determined by coulometric Karl Fischer titration on Metrohm 831 KF Coulometer. Rotations were recorded on polAAr31 polarimeter, at 20 °C in chloroform (compounds **5–14**) or methanol (compounds **15–17**). Concentrations are given in percentage (g/100 mL).

Table 1
Antibacterial activity of new compounds **5**–**17** in comparison to amoxycillin

Core structure	Compound[a]	R_1	R_2	MIC (μg/mL) Bacterial strains[b]					
				1	2	3	4	5	6
	**5**	Ph	COOBn			>128			
	6	Me	COOBn			>128			
	7	H	COOBn			64–128			
	8	H	Bn			64–128			
	9	Me	Me			>128			
	*trans*-**10**	Ph	COOBn	16	8	64	32	>128	>128
	trans-**15**		COOH			>128			
	trans-**11**	Me	COOBn	64	64	32–64	64–128	>128	>128
	cis-**11**								
	trans-**16**		COOH	>128					
	trans-**12**	H	COOBn	32–64	64	32	64	>128	>128
	cis-**12**								
	trans-**17**		COOH	>128					
	trans-**13**	H	Bn	64	64–128	16	128	>128	>128
	cis-**13**								
	trans-**14**	Me	Me			64–128			
	cis-**14**								
	Amoxycillin			4	4	0.5	1	0.5	0.015

[a] Stability at testing conditions (pH 7.4; $T = 35$ °C) of all compounds was confirmed by LC/MS (Supplementary data).
[b] *E. faecalis* ATCC 29212 (1); *S. pneumoniae* ATCC 6305 (2); *M. catarrhalis* ATCC 23246 (3); *S. aureus* ATCC 29213 (4); *E. coli* ATCC 25922 (5); *H. influenzae* ATCC 49247 (6).

3.2. Chemistry

3.2.1. General procedure for the preparation of compounds 5–9

6-PhtPA (10.0 g, 28.9 mmol) was suspended in dichloromethane (DCM; 300 mL) and dissolved by the addition of triethylamine (TEA; 4.0 mL, 28.9 mmol) with stirring at room temperature. The solution was cooled to 0 °C and ethyl chloroformate (2.62 mL, 32.4 mmol) in DCM (150 mL) was added. The reaction mixture was stirred for 1/2 h at 0 °C. Corresponding amine (29.9 mmol) was dissolved in DCM (40 mL) (with the addition of eqTEA, if necessary) and added drop wise to the reaction mixture during 1/2 h at 0 °C. After 2 h of stirring at 0 °C, water (500 mL) was added to the reaction mixture and the layers were separated. Organic layer was washed with water (500 mL), dried over sodium sulfate and solvent was evaporated under reduced pressure.

3.2.1.1. (*S*)-Benzyl 2-((*2S,5R,6R*)-6-(1,3-dioxoisoindolin-2-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxamido)-2-phenylacetate (5). Yield 76%; mp: 122–125 °C; $[\alpha]_D$ = +360.1 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3362, 1798, 1778, 1729, 1681, 1504, 1385, 1202, 1172, 720, 698; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.42 (s, Me), 1.67 (s, Me), 4.70 (s, C_2–H), 5.14 and 5.17 (2d, CH_2, *J* = 12.2 Hz), 5.53 (d, CH, *J* = 6.6 Hz), 5.56 (d, C_5–H, *J* = 4.1 Hz), 5.75 (d, C_6–H, *J* = 4.0 Hz), 7.22–7.33 (m, 5H-Ph), 7.39–7.43 (m, 5H-Ph), 7.88–7.93 (m, 4H-Pht), 9.19 (d, NH, *J* = 6.4 Hz); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 27.2, 31.4, 56.3, 58.9, 65.8, 66.4, 67.1, 69.7, 123.6, 127.7, 127.8, 127.9, 128.3, 128.5, 128.8, 130.9, 135.0, 135.5, 166.2, 166.7, 167.7, 170.0; HRMS calcd for $C_{31}H_{27}N_3O_6S$ [M+Na]$^+$: 592.1518. Found: 592.1546.

3.2.1.2. (*S*)-Benzyl 2-((*2S,5R,6R*)-6-(1,3-dioxoisoindolin-2-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)propanoate (6). Yield 94%; mp: 65–70 °C; $[\alpha]_D$ = +290.9 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3390, 1800, 1780, 1727, 1674, 1519, 1386, 1201, 720; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.37 (d, Me, *J* = 7.3 Hz), 1.42 (s, Me), 1.68 (s, Me), 4.43 (pent, CH, *J* = 7.2 Hz), 4.53 (s, C_2–H), 5.16 and 5.19 (2d, CH_2, *J* = 12.5 Hz), 5.57 (d, C_5–H, *J* = 4.0 Hz), 5.76 (d, C_6–H, *J* = 4.0 Hz), 7.36–7.40 (m, 5H-Ph), 7.90–7.95 (m, 4H-Pht), 8.78 (d, NH, *J* = 7.1 Hz); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 16.5, 26.7, 30.6, 47.2, 58.2, 65.1, 65.6, 66.2, 70.0, 123.1, 127.3, 127.6, 128.0, 130.4, 134.5, 135.4, 165.7, 166.3, 167.6, 171.5; HRMS calcd for $C_{26}H_{25}N_3O_6S$ [M+Na]$^+$: 530.1362. Found: 530.1352.

3.2.1.3. Benzyl 2-((*2S,5R,6R*)-6-(1,3-dioxoisoindolin-2-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)acetate (7). Yield 91%; mp: 93–97 °C; $[\alpha]_D$ = +247.6 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3553, 1790, 1768, 1727, 1675, 1540, 1384, 1313, 1201, 719; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.43 (s, Me), 1.66 (s, Me), 4.00 (d, CH_2, *J* = 5.8 Hz), 4.48 (s, C_2–H), 5.15 (s, CH2), 5.55 (d, C_5–H, *J* = 4.0 Hz), 5.74 (d, C_6–H, *J* = 4.1 Hz), 7.34–7.39 (m, 5H-Ph), 7.89–7.94 (m, 4H-Pht), 8.79 (t, NH, *J* = 5.8 Hz); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 27.2, 30.4, 40.6, 58.2, 65.3, 66.3, 66.3, 70.7, 123.6, 128.1, 128.1, 128.4, 130.8, 135.0, 135.7, 166.2, 167.4, 168.4, 169.3; HRMS calcd for $C_{25}H_{23}N_3O_6S$ [M+Na]$^+$: 516.1205. Found: 516.1207; Anal. Calcd for $C_{25}H_{23}N_3O_6S \times 1.5H_2O$: C, 57.68; H, 5.03; N, 8.07; O, 23.05; S, 6.16. Found: C, 57.95; H, 5.21; N, 7.95; S, 6.22; KF_q (water): 5.25% (calcd: 5.19%).

3.2.1.4. (*2S,5R,6R*)-*N* -Benzyl-6-(1,3-dioxoisoindolin-2-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide (8). Yield 91%; mp: 75–79 °C; $[\alpha]_D$ = +265.5 (*c* 0.1, chloroform).[22]

3.2.1.5. (*2S,5R,6R*)-6-(1,3-Dioxoisoindolin-2-yl)-*N*-isopropyl-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxamide (9). Yield 85%; mp: 85–90 °C; $[\alpha]_D$ = +330.7 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3367, 2972, 1800, 1780, 1727, 1654, 1526, 1387, 719; ^{1}H NMR/ppm (300 MHz, DMSO-δ_6): 1.09 (d, Me, *J* = 6.2 Hz), 1.11 (d, Me, *J* = 6.4 Hz), 1.42 (s, Me), 1.67 (s, Me), 3.88 (m, 1H), 4.40 (s, C_2–H), 5.56 (d, C_5–H, *J* = 3.9 Hz), 5.74 (d, C_6–H, *J* = 3.9 Hz), 7.88–7.95 (m, 4H-Pht), 8.12 (d, NH, *J* = 7.7 Hz); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 22.6, 22.7, 27.5, 31.8, 41.0, 59.2, 66.0, 67.3, 71.0, 124.0, 131.4, 135.5, 166.2, 166.7, 168.4; HRMS calcd for $C_{19}H_{21}N_3O_4S$ [M+Na]$^+$: 410.1150. Found: 410.1151.

3.2.2. General procedure for the preparation of compounds 10–14

Corresponding compound **5–9** (1.0 mmol) was dissolved in DCM (20 mL) and sulfuryl chloride (1.0 mmol, 82.9 μL) was added. The reaction mixture was stirred for 1 h at room temperature and water was added (20 mL). The layers were separated and organic layer was washed with water (20 mL), dried over sodium sulfate and solvent was evaporated under reduced pressure. Diastereomeric mixtures **10–14** were separated by column chromatography (toluene/EtOA *c* 3:1) of the residue after evaporation to obtain *cis*- and *trans*-diastereomers.

3.2.2.1. (*S*)-Benzyl 2-((*S*)-4-((*2S,3R*)-2-chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)-2-phenylacetate (*trans*-10). Yield 94%; mp 147–150 °C; $[\alpha]_D$ = −51.9 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3375, 2921, 1797, 1720, 1668, 1497, 1394, 1169, 715, 698; ^{1}H NMR/ppm (300 MHz, $CDCl_3$): 1.38 (s, Me), 1.67 (s, Me), 4.43 (s, 1H), 5.32 (s, CH_2), 5.57 (d, C_3–H, *J* = 1.8 Hz), 6.19 (d, C_2–H, *J* = 1.8 Hz), 6.22 (s, CH), 7.25–7.44 (m, 10H, Ph x 2), 7.75–7.94 (m, 4H-Pht); ^{13}C NMR/ppm (75 MHz, $CDCl_3$): 22.1, 25.4, 54.6, 59.6, 64.3, 65.3, 67.4, 70.7, 123.9, 128.3, 128.4, 128.4, 128.5, 128.6, 128.8, 129.1, 129.2, 131.3, 131.4, 132.8, 134.7, 161.0, 166.2, 166.4, 168.8; HRMS calcd for $C_{31}H_{26}N_3O_6SCl$ [M+Na]$^+$: 626.1129. Found: 626.1130.

3.2.2.2. (*S*)-Benzyl 2-((*S*)-4-((*2R,3R*)-2-chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)propanoate (*cis*-11). Yield 7%; mp 81–84 °C; $[\alpha]_D$ = +38.1 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3488, 2930, 1794, 1775, 1728, 1686, 1456, 1391, 1310, 1202, 927, 722; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.38 (d, Me, *J* = 7.1 Hz), 1.55 (s, Me), 1.64 (s, Me), 4.77 (s, 1H), 5.05 (q, CH, *J* = 7.1 Hz), 5.15 and 5.18 (2d, CH_2, *J* = 12.4 Hz), 5.89 (d, C_3–H, *J* = 4.2 Hz), 6.73 (d, C_2–H, *J* = 4.2 Hz), 7.35–7.40 (m, 5H-Ph), 7.93–8.00 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 15.2, 23.9, 25.0, 51.9, 55.7, 59.9, 64.4, 67.2, 72.2, 124.4, 128.4, 128.7, 129.0, 131.3, 135.8, 136.0, 163.6, 167.4, 170.2; HRMS calcd for $C_{26}H_{24}N_3O_6SCl$ [M+Na]$^+$: 564.0972. Found: 564.0972.

3.2.2.3. (*S*)-Benzyl 2-((*S*)-4-((*2S,3R*)-2-chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)propanoate (*trans*-11). Yield 63%; mp 68–70 °C; $[\alpha]_D$ = −45.0 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3375, 2925, 1796, 1775, 1726, 1695, 1456, 1395, 1310, 1103, 962, 723; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.37 (d, Me, *J* = 7.1 Hz), 1.59 (s, Me), 1.59 (s, Me), 4.51 (s, 1H), 5.05 (q, CH, *J* = 7.2 Hz), 5.16 and 5.18 (2d, CH_2, *J* = 12.5 Hz), 5.58 (d, C_3–H, *J* = 1.6 Hz), 6.43 (d, C_2–H, *J* = 1.6 Hz), 7.38–7.40 (m, 5H-Ph), 7.90–7.96 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 14.7, 21.8, 25.8, 50.9, 53.9, 63.2, 64.5, 66.6, 71.7, 123.6, 128.1, 128.2, 128.4, 131.2, 135.0, 135.4, 161.2, 166.1, 166.2, 169.7; HRMS calcd for $C_{26}H_{24}N_3O_6SCl$ [M+Na]$^+$: 564.0972. Found: 564.0983.

3.2.2.4. Benzyl 2-((*S*)-4-((*2R,3R*)-2-chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)acetate (*cis*-12). Yield 3%; mp: 136–139 °C; $[\alpha]_D$ = +26.7 (*c*

0.1, chloroform); IR/cm^{-1} (KBr): 3420, 2971, 1794, 1750, 1726, 1700, 1457, 1395, 1193, 1104, 963, 725; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.63 (s, Me), 1.66 (s, Me), 4.29 and 4.34 (2d, CH_2, J = 17.9 Hz), 4.82 (s, 1H), 5.16 and 5.19 (2d, CH_2, J = 12.4 Hz), 5.90 (d, C_3–H, J = 4.1 Hz), 6.78 (d, C_2–H, J = 4.3 Hz), 7.34–7.39 (m, 5H-Ph), 7.93–8.00 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 23.9, 24.6, 45.4, 55.4, 59.4, 63.2, 66.5, 71.4, 123.8, 128.1, 128.2, 128.4, 130.8, 135.3, 135.4, 163.1, 166.2, 167.1, 167.4; HRMS calcd for $C_{25}H_{22}N_3O_6SCl$ [M+Na]$^+$: 550.0816. Found: 550.0823.

3.2.2.5. Benzyl 2-((*S*)-4-((*2S,3R*)-2-chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl -3-oxoisothiazolidin-2-yl)acetate (*trans*-12). Yield 60%; mp: 88–90 °C; $[\alpha]_D$ = −69.8 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 2968, 1795, 1750, 1726, 1700, 1456, 1396, 1192, 1104, 963, 725; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.64 (s, Me), 1.67 (s, Me), 4.22 and 4.36 (d, CH_2, J = 17.7 Hz), 4.58 (s, 1H), 5.18 (s, CH_2), 5.57 (d, C_3–H, J = 1.6 Hz), 6.43 (d, C_2–H, J = 1.6 Hz), 7.37–7.39 (m, 5H-Ph), 7.89–7.96 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 22.4, 25.6, 45.4, 54.2, 63.0, 64.3, 66.4, 71.6, 123.6, 128.1, 128.2, 128.4, 131.2, 135.0, 135.5, 161.3, 166.2, 166.4, 167.5; HRMS calcd for $C_{25}H_{22}N_3O_6SCl$ [M+Na]$^+$: 550.0816. Found: 550.0814.

3.2.2.6. 2-((*2R,3R*)-1-((*S*)-2-Benzyl-5,5-dimethyl-3-oxoisothiazolidin-4-yl)-2-chloro-4-oxoazetidin-3-yl)isoindoline-1,3-dione (*cis*-13). Yield 1%; mp 203–206 °C; $[\alpha]_D$ = +17.3 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3309, 2972, 1789, 1772, 1724, 1686, 1456, 1389, 1202, 723; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.50 (s, Me), 1.62 (s, Me), 4.56 and 4.60 (2d, CH_2, J = 15.0 Hz), 4.88 (s, 1H), 5.92 (d, C_3–H, J = 4.3 Hz), 6.85 (d, C_2–H, J = 4.2 Hz), 7.29–7.38 (m, 5H-Ph), 7.93–8.00 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 24.0, 24.7, 47.7, 54.6, 59.3, 63.6, 71.6, 123.8, 127.9, 128.1, 128.5, 130.8, 135.3, 135.7, 163.0, 166.6; HRMS calcd for $C_{23}H_{20}N_3O_6SCl$ [M+Na]$^+$: 492.0761. Found: 492.0762.

3.2.2.7. 2-((*2S,3R*)-1-((*S*)-2-Benzyl-5,5-dimethyl-3-oxoisothiazolidin-4-yl)-2-chloro-4-oxoazetidin-3-yl)isoindoline-1,3-dione (*trans*-13). Yield 54%; mp 70–72 °C; $[\alpha]_D$ = −43.3 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 2971, 1793, 1770, 1727, 1689, 1456, 1395, 1104, 722; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.55 (s, Me), 1.56 (s, Me), 4.45 and 4.67 (2d, CH_2, J = 14.9 Hz), 4.62 (s, 1H), 5.59 (d, C_3–H, J = 1.6 Hz), 6.42 (d, C_2–H, J = 1.6 Hz), 7.31–7.37 (m, 5H-Ph), 7.90–7.96 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 22.5, 25.8, 47.8, 53.3, 63.7, 64.2, 71.6, 123.6, 127.8, 128.3, 128.5, 131.2, 135.0, 135.7, 161.3, 165.9, 166.2; HRMS calcd for $C_{23}H_{20}N_3O_6SCl$ [M+Na]$^+$: 492.0761. Found: 492.0770.

3.2.2.8. 2-((*2R,3R*)-2-Chloro-1-((*S*)-2-isopropyl-5,5-dimethyl-3-oxoisothiazolidin-4-yl)-4-oxoazetidin-3-yl)isoindoline-1,3-dione (*cis*-14). Yield 8%; mp 102–105 °C; $[\alpha]_D$ = +31.2 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3490, 2972, 1795, 1782, 1726, 1682, 1467, 1395, 1103, 720; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.09 (d, Me, J = 6.7 Hz), 1.13 (d, Me, J = 6.5 Hz), 1.54 (s, Me), 1.65 (s, Me), 4.43 (sept, CH, J = 6.6 Hz), 4.76 (s, 1H), 5.89 (d, C_3–H, J = 4.1 Hz), 6.78 (d, C_2–H, J = 4.2 Hz), 7.92–7.98 (m. 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 19.3, 20.5, 23.7, 24.2, 45.0, 54.2, 59.3, 64.3, 71.7, 123.8, 130.8, 135.8, 163.1, 165.7, 166.4; HRMS calcd for $C_{19}H_{20}N_3O_6SCl$ [M+Na]$^+$: 444.0761. Found: 444.0740.

3.2.2.9. 2-((*2S,3R*)-2-Chloro-1-((*S*)-2-isopropyl-5,5-dimethyl-3-oxoisothiazolidin-4-yl)-4-oxoazetidin-3-yl)isoindoline-1,3-dione (*trans*-14). Yield 42%; mp 116–119 °C; $[\alpha]_D$ = −81.4 (*c* 0.1, chloroform); IR/cm^{-1} (KBr): 3447, 2972, 1794, 1780, 1726, 1683, 1467, 1396, 1104, 721; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.11 (d, Me, J = 6.5 Hz), 1.13 (d, Me, J = 6.6 Hz), 1.60 (s, Me), 1.60 (s, Me), 4.43 (m, CH), 4.45 (s, CH), 5.56 (d, C_3–H, J = 1.5 Hz), 6.44 (d, C_2–H, J = 1.4 Hz), 7.90–7.95 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 19.7, 19.9, 21.7, 26.1, 44.8, 53.2, 64.1, 64.5, 71.7, 123.6, 131.2, 135.0, 161.2, 165.1, 166.2; HRMS calcd for $C_{19}H_{20}N_3O_6SCl$ [M+Na]$^+$: 444.0761. Found: 444.0756.

3.2.3. General procedure for the preparation of compounds 15–17

Corresponding compound *trans*-**10–12** (0.19 mmol) was dissolved in DCM (10 mL) and cooled to 0 °C. Aluminium chloride (150 mg; 1.12 mmol) was dissolved in nitromethane (5 mL) and added drop wise to the DCM solution. The reaction mixture was stirred for 1/2 h at 0 °C. EtOAc (10 mL) was added and the solution acidified with HCl, 0.1 M (15 mL). The layers were separated and organic layer was washed with water (20 mL), dried over sodium sulfate and solvent evaporated under reduced pressure. Compounds *trans*-**15–17** were purified by acid–base extractions in water/DCM system by alkalizing with $NaHCO_3$, satd to pH 8 and acidifying with HCl, 1.2 M to pH 2.

3.2.3.1. (*S*)-2-((*S*)-4-((*2S ,3R*)-2-Chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)-2-phenylacetic acid (*trans*-15). Yield 65%; mp 111–114 °C; $[\alpha]_D$ = −57.8 (*c* 0.1, methanol); IR/cm^{-1} (KBr): 3444, 2927, 1778, 1731, 1668, 1628, 1615, 1361, 1296, 703; ^{1}H NMR/ppm (300 MHz, $CDCl_3$): 1.39 (s, Me), 1.76 (s, Me), 4.35 (s, 1H), 5.61 (d, C_3–H, J = 1.6 Hz), 6.24 (s, 1H, CH(Ph)), 6.30 (d, C_2–H, J = 1.6 Hz), 7.40 (s, 5H-Ph), 7.77–7.91 (m, 4H-Pht); ^{13}C NMR/ppm (75 MHz, $CDCl_3$): 22.3, 25.2, 54.5, 59.7, 64.6, 65.1, 70.8, 123.6, 128.5, 128.6, 128.7, 129.0, 129.2, 131.3, 131.5, 133.0, 161.1, 166.1, 166.4, 170.7; HRMS calcd for $C_{24}H_{20}N_3O_6SCl$ [M+Na]$^+$: 536.0659. Found: 536.0655.

3.2.3.2. (*S*)-2-((*S*)-4-((*2S ,3R*)-2-Chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)-propanoic acid (*trans*-16). Yield 55%; mp 83–86 °C; $[\alpha]_D$ = −43.4 (*c* 0.1, methanol); IR/cm^{-1} (KBr): 3381, 2927, 1797, 1728, 1694, 1456, 1395, 1103, 722; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.32 (d, Me, J = 7.2 Hz), 1.59 (s, Me), 1.67 (s, Me), 4.53 (s, 1H), 4.88 (q, CH, J = 7.2 Hz), 5.57 (d, C_3–H, J = 1.4 Hz), 6.44 (d, C_2–H, J = 1.3 Hz), 7.90–7.96 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 14.7, 22.0, 25.5, 50.8, 53.9, 63.6, 64.4, 71.7, 123.6, 131.2, 135.0, 161.2, 166.0, 166.2, 171.4; HRMS calcd for $C_{19}H_{18}N_3O_6SCl$ [M+Na]$^+$: 474.0503. Found: 474.0506.

3.2.3.3. 2-((*S*)-4-((*2S,3R*)-2-Chloro-3-(1,3-dioxoisoindolin-2-yl)-4-oxoazetidin-1-yl)-5,5-dimethyl-3-oxoisothiazolidin-2-yl)acetic acid (*trans*-17). Yield 29%; mp 84–88 °C; $[\alpha]_D$ = −56.9 (*c* 0.1, methanol); IR/cm^{-1} (KBr): 3375, 2929, 1795, 1727, 1692, 1396, 1105, 723; ^{1}H NMR/ppm (600 MHz, DMSO-δ_6): 1.65 (s, Me), 1.69 (s, Me), 3.98 and 4.21 (d, CH_2, J = 17.7 Hz), 4.56 (s, 1H), 5.57 (d, C_3–H, J = 1.5 Hz), 6.43 (d, C_2–H, J = 1.4 Hz), 7.90–7.97 (m, 4H-Pht); ^{13}C NMR/ppm (150 MHz, DMSO-δ_6): 22.4, 25.5, 45.4, 54.1, 63.1, 64.2, 71.6, 123.6, 131.2, 135.0, 161.3, 166.2, 166.4, 168.9; HRMS calcd for $C_{18}H_{16}N_3O_6SCl$ [M+Na]$^+$: 460.0346. Found: 460.0354.

3.3. In vitro antibacterial screen

The antibacterial testing was performed by determining the MICs using the broth microdilution method as recommended by the CLSI (formerly the National Committee for Clinical Laboratory Standards)[26] with minor modifications. Cation-adjusted Mueller–Hinton broth (Difco Laboratories) was used and was supplemented with 5% horse serum for *S. pneumoniae* while HTM (Haemophilus Test Medium) was used for *H. influenzae*. Suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending the colonies from overnight agar cultures in 2 mL of sterile saline. The suspensions were further diluted in

appropriate media to obtain a final inoculum of 1×10^4 CFU/well. The trays were incubated overnight in ambient air at 35 °C.

Supplementary data

Supplementary data (copies of ^{1}H and ^{13}C NMR spectra of all the compounds, 2D NMR spectra (COSY, HMQC, HMBC) of compound *cis*-**13** and LC/MS chromatograms of all the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.045.

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