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ARTICLE TYPE

Supplementary information *In vitro* anticancer activity and SAR of triazolyl aminoacyl(peptidyl) penicillins

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Experimental

1. Chemistry

Solvents were analytical grade or were purified by standard procedures prior to use. Infrared spectra (IR) were recorded on a Shimadzu Prestige 21 spectrophotometer and only partial spectral data are listed. The ^{1}H and ^{13}C NMR spectra were acquired in the specified solvent, in a Bruker Avance spectrometer (300 and 75 MHz for ^{1}H and ^{13}C, respectively), with tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) are reported in ppm downfield from TMS and coupling constants (*J*) are expressed in hertz. The high resolution mass spectra were obtained with a Bruker MicroTOF-Q II instrument (Bruker Daltonics, Billerica, MA). Detection of the ions was performed in electrospray ionization, positive ion mode. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh). Elution was carried out with hexane-EtOAc mixtures, under positive pressure and employing gradient of solvent polarity techniques.

1.1. (2S)-2-(1-(((2S)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-phenylpropanoic acid (6b)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.53 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 3.26 (m, 2H, CH_2), 4.35 (m, 1H, CH_2), 4.47 (m, 1H, CH), 4.63 (1m, 1H, CH), 5.00 (m, 1H, CH), 5.63 (s, 1H, CH), 7.16-7.29 (m, 5H, ArH), 7.44 (d, *J*=7.6 Hz, 1H, NH), 8.33 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 24.4 (CH_3), 33.2 (CH_3), 37.4 (CH_2), 48.3 (CH_2), 53.4 (CH), 58.6 (C), 63.7 (C), 67.8 (CH), 78.3 (CH), 127.2 (CH), 128.7 (CH), 129.3 (CH), 135.8 (C), 142.6 (C), 160.1 (CO), 165.7 (CO), 173.9 (CO). **IR:** (film) 1789 cm^{-1} (β-lactam), 1740 cm^{-1} (CO_2H), 1670 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{20}H_{21}Br_2N_5NaO_4S$ $[M+Na]^+$: 607.9579; found 607.9573.

1.2. (2S)-2-(1-(((2S)-6,6-Dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-methylbutanoic acid (6c)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (m, 3H, CH_3), 1.06 (m, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 2.34 (m, 1H, CH), 4.37-4.50 (m, 2H, CH and CH_2), 4.66-4.71 (m, 2H, CH and CH_2), 5.68 (s, 1H, CH), 7.67 (d, 1H, NH, *J*= 8.4 Hz) 8.42 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 17.9 (CH_3), 19.2 (CH_3), 24.5 (CH_3), 30.9 (CH), 33.3 (CH_3), 48.3 (CH_2), 57.4 (C), 58.6 (CH), 63.7 (C), 67.9 (CH), 78.3 (CH), 126.8 (CH), 142.8(C), 159.9 (CO), 160.3 (CO), 165.7 (CO). IR: (film) 1789 cm^{-1} (β-lactam), 1730 cm^{-1} (CO_2H), 1665 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{16}H_{21}Br_2N_5NaO_4S$ $[M+Na]^+$: 559.9579; found 559.95732.

1.3. Methyl 2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)acetate (7a)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 4.25 (d, *J*= 5.64 Hz, 2H, CH_2), 4.37 (m, 1H, CH_2), 4.42 (m, 1H, CH), 4.70 (dd, J_1= 12.6 Hz, J_2= 3 Hz, 1H, CH_2), 5.64 (s, 1H, CH), 7.63 (t, *J*= 5.64 Hz, 1H, NH), 8.23 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 24.5 (CH_3), 33.2 (CH_3), 40.9 (CH_2), 48.3 (CH_2), 52.5 (CH_3), 58.5(C), 63.6 (C), 67.9 (CH), 78.4 (CH), 126.1 (CH), 143.1 (C), 159.9 (CO), 165.6 (CO), 169.7 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1747 cm^{-1} (CO ester), 1672 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd. for $C_{14}H_{17}Br_2N_5O_4S$ $[M+Na]^+$: 531.9265; found 531.9260.

1.4. (2S)-Methyl 2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1H-1,2,3-triazole-4-carboxamido)-3-methylbutanoate (7c).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.00 (d, *J*= 3.4 Hz, 3H, CH_3), 1.02 (d, *J*= 3.4 Hz, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 2.28 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 4.31 (m, 1H, CH_2), 4.41 (m, 1H, CH), 4.70 (m, 1H, CH_2), 4,72 (m, 1H, CH), 5.68 (s, 1H, CH), 7.57 (d, *J*= 9Hz, 1H, NH) 8.32 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 300 MHz)** δ 17.9 (CH_3), 19.1(CH_3), 24.5 (CH_3), 31.4 (CH), 33.3 (CH_3), 48.3 (CH_2), 52.3 (OCH_3), 57.1 (CH_3), 58.6(C), 63.5 (C), 67.9 (CH), 78.4 (CH), 126.4 (CH), 143.2 (C), 159.7 (CO), 165.6 (CO), 171.7 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1666 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{15}H_{19}Br_2N_5NaO_4S$ $[M+Na]^+$: 573.9735; found 573.9729.

1.5. (2S)-Methyl 2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-phenylpropanoate (7b).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (s, 3H, CH_3), 1.59 (s, 3H,

CH_3), 3.22 (m, 2H, CH_2), 3.73 (s, 3H, OCH_3), 4.38 (m, 1H, CH) 4.68 (dd, J_1=12.6 Hz, J_2= 2.7Hz, 2H, CH_2), 5.05 (m, 1H, CH), 5.64 (s, 1H, CH), 7.16-7.31 (m, 5H, ArH), 7.54 (d, *J*=8.46 Hz, 1H, NH), 8.19 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 24.5 (CH_3), 33.2 (CH_3), 38.2 (CH_2), 48.3 (CH_2), 52.4 (CH_3), 53.2 (CH), 58.5 (C), 63.6 (C), 67.9 (CH), 78.4 (CH), 126.1 (CH), 127.2 (CH), 128.6 (CH), 129.2 (CH), 135.7 (C), 143.0 (C), 159.34 (CO), 165.5 (CO), 171.3 (CO). **IR:** (film) 1789 cm^{-1} (β-lactam), 1745 cm^{-1} (CO ester), 1672 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{21}H_{23}Br_2N_5O_4S$ $[M+H]^+$: 599.9915; found 599.9910.

1.6. (2S)-Methyl 2-(2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)acetamido)-3-phenylpropanoate (7d)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 3.11 (ddd, J_1= 21 Hz, J_2= 14 Hz, J_3= 6 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 4.11 (d, *J*= 5.7 Hz, 2H, CH_2), 4.36 (m, 1H, CH_2), 4.46 (m, 1H, CH), 4.69 (dd, J_1= 13 Hz, J_2= 3.8 Hz, 2H, CH_2), 4.89 (m, 1H, CH), 5.65 (s, 1H, CH), 6.70 (d, *J*= 7.6 Hz, 1H, NH), 7.07-7.22 (m, 5H , ArH), 7.79 (t, *J*= 5,7 Hz, 1H, NH), 8.25 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 24.5 (CH_3), 33.2 (CH_3), 37.8 (CH_2), 42.9 (CH_2), 48.4 (CH_2), 52.4 (CH_3), 53.2 (CH), 58.5 (C), 63.6 (C), 67.9 (CH), 78.4 (CH), 126.2 (CH), 127.1 (CH), 128.6 (CH), 129.2 (CH), 135.7 (C), 142.9 (C), 160.2 (CO), 165.6 (CO), 168.14 (CO), 171.8 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1690 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{23}H_{26}Br_2N_6NaO_5S$ $[M+Na]^+$: 678.9944; found 678.9943.

1.7. (2S)-Methyl 2-((2S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (7e).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, *J*= 6.2 Hz, 3H, CH_3), 0.93 (d, *J*= 6.2 Hz, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.59-1.76 (m, 2H, CH_2), 1.61 (s, 3H, CH_3), 2.97 (dd, J_1= 14 Hz, J_2= 6,54 Hz, 1H, CH_2), 3,10 (dd, J_1= 14 Hz, J_2= 5.4 Hz 1H, CH_2), 3.76 (s, 3H, OCH_3), 4.33 (dd, J_1= 14 Hz, J_2=10.9 Hz, 1H, CH_2), 4.51 (dd, J_1= 11 Hz, J_2=3.4 Hz, 1H, CH), 4.59-4.67 (m, 1H, CH), 4.74-4,86 (m, 2H, CH and CH_2), 5.71 (s, 1H, CH), 6.46 (d, *J*= 8.4 Hz, 2H, ArH), 6.76 (d, *J*= 8 Hz, 1H, NH), 6.84 (d, *J*= 8.4 Hz, 2H, ArH), 7.37 (d, *J*= 8.4 Hz, 1H, NH), 8.22 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 21.9 (CH_3), 22.9 (CH_3), 24.7 (CH_3), 33.2 (CH_3), 36.5 (CH_2), 39.9 (CH_2), 48.4 (CH_2), 51.1 (CH), 52.5 (CH), 53.0 (CH) 58.0 (C), 63.6 (C), 68.2 (CH), 78.5 (CH), 115.3 (CH), 126.9 (CH), 130.3 (CH), 142.8 (C), 155.0 (C), 159.8 (CO), 166.7 (CO), 170.8 (CO), 171.9 (CO). **IR:** (film) 1789 cm^{-1} (β-lactam), 1747 cm^{-1} (CO ester), 1670 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{27}H_{34}Br_2N_6NaO_6S$ $[M+Na]^+$: 751.05195; found: 751.05135.

1.8. (2S)-Methyl 2-((2S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanamido)-3-phenylpropanoate (7f).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, *J*= 5.6 Hz, 3H, CH_3), 0.92 (d, *J*= 5.6 Hz, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.59-1.76 (m, 3H, CH and CH_2), 3.04 (dd, J_1= 13.7 Hz, J_2= 6.3 Hz, 1H, CH_2), 3.14 (dd, J_1= 13.7 Hz, J_2= 5.7 Hz, 1H, CH_2), 3.69 (s, 3H, OCH_3), 4.37 (dd, J_1= 13.3 Hz, J_2= 10 Hz, 1H, CH_2), 4.49 (dd, J_1= 10 Hz, J_2= 3.9, 1H, CH), 4.60 (m, 1H, CH), 4.70 (dd, J_1= 13.3Hz, J_2= 3.9, 1H, CH_2), 4.87 (m, 1H, CH), 5.66 (s, 1H, CH), 6.75 (d, *J*= 8 Hz, 1H, NH), 7.04-7.15 (m, 5H, ArH), 7.38 (d, *J*= 8.2 Hz, 1H, NH), 8.26 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 21.9 (CH_3), 22.9 (CH_3), 24.5 (CH), 24.6 (CH_3), 33.3 (CH_3), 37.9 (CH_2), 40.7 (CH_2), 48.4 (CH_2), 51.3 (CH), 52.3 (CH), 53.2 (CH) 58.5 (C), 63.7 (C), 67.9 (CH), 78.4 (CH), 126.2 (CH), 127.0 (CH), 128.5 (CH), 129.2 (CH), 135.6 (C), 143.0 (C), 159.7 (CO), 165.6 (CO), 171.0 (CO), 171.8 (CO). **IR:** (film) 1797 cm^{-1} (β-lactam), 1744 cm^{-1} (CO ester), 1670 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{27}H_{34}Br_2N_6NaO_5S$ $[M+Na]^+$: 735.05704; found: 735.05518.

1.9. (2S)-Methyl 2-((2S,3S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-methylpentanamido)-3-phenylpropanoate (7h).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, *J*=7,2 Hz, 3H, CH_3), 0.93 (d, *J*= 6.6 Hz, 3H, CH_3), 1.13 (m, 1H, CH_2), 1.48 (m, 1H, CH_2), 1.55 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.96 (m, 1H, CH), 3.05 (dd, J_1= 13.8 Hz, J_2= 6.3, 1H, CH_2), 3.13 (dd, J_1= 13.8 Hz, J_2= 5.9, 1H, CH_2), 3.68 (s, 3H, OCH_3), 4.39 (m, 1H, CH_2), 4.51 (m, 2H, CH), 4.73 (m, 1H, CH_2), 4.90 (m, 1H, CH), 5.66 (s, 1H, CH), 6.72 (d, *J*= 8 Hz, 1H, NH), 7.06-7.21 (m, H, ArH), 7.57 (d, *J*= 9.2 Hz, 1H, NH), 8.35 (m, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 11.1 (CH_3), 15.5 (CH_3), 24.5 (CH_3), 24.8 (CH_2), 33.1 (CH_3), 37.2 (CH), 38.0 (CH_2), 48.3 (CH_2), 52.4 (OCH_3), 53.1 (CH), 57.4 (CH), 58.2 (C), 63.7 (C), 67.7 (CH), 78.4 (CH), 126.2 (CH), 127.0 (C), 128.5 (CH), 129.2 (CH), 135.6 (CH), 143.2 (C), 159.6 (CO), 165.5 (CO), 170.3 (CO), 171.9 (CO). **IR:** (film) 1795 cm^{-1} (β-lactam), 1745 cm^{-1} (CO ester), 1643 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{27}H_{34}Br_2KN_6O_5S$ $[M+K]^+$: 751.03097; found: 751.02874.

1.10. (2S)-Methyl 2-((2S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-methylbutanamido)-3-phenylpropanoate (7i).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, *J*= 7.2 Hz, 6H, CH_3), 1.55 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 2.19 (m, 1H, CH), 3.05 (dd, J_1= 14 Hz, J_2= 6.5Hz, 1H, CH_2), 3.12 (dd, J_1= 14 Hz, J_2= 6 Hz, 1H, CH_2), 3.68 (s, 3H, OCH_3), 4.37 (dd, J_1= 13.4 Hz, J_2= 10.3 Hz, 1H, CH_2), 4.52 (m, 1H, CH), 4.53 (m, 1H, CH), 4.71 (dd, J_1= 13.4 Hz, J_2= 4.2 Hz, 1H, CH_2), 4.91 (m, 1H, CH), 5.66 (s, 1H, CH), 6.79 (d, *J*= 8 Hz, 1H, NH), 7.07-7.18 (m, 5H, ArH), 7.60 (d, *J*= 9 Hz, 1H, NH), 8.36 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 18.0 (CH_3), 19.2 (CH_3), 24.4 (CH_3), 31.1 (CH), 33.2 (CH_3), 38.0 (CH_2), 48.4 (CH_2), 52.3 (OCH_3), 53.1 (CH) 58.1 (CH), 58.6 (C), 63.7 (C), 67.7 (CH), 78.4 (CH), 126.3 (CH), 127.1 (CH), 128.5 (CH), 129.2 (CH), 135.6 (C), 143.2 (C), 159.7 (CO), 165.6 (CO), 170.3 (CO), 171.8 (CO). **IR:** (film) 1789 cm^{-1} (β-lactam), 1737 cm^{-1} (CO ester), 1645 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{26}H_{32}Br_2N_6NaO_5S$ $[M+Na]^+$: 721.04139; found: 721.04279.

1.11. (2S)-Methyl 2-((2S)-1-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carbonyl)pyrrolidine-2-carboxamido)-3-phenylpropanoate (7j).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.58 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.9 (m, 2H, CH_2), 2.32 (m, 2H, CH_2), 2.91-3.20 (m, 2H, CH_2), 3.70 (s, 3H, OCH_3), 3.73 (m, 2H, CH_2), 4.07 (m, 2H, CH_2), 4.40 (m, 1H, CH), 4.44 (m, 1H, CH_2), 4.67 (m, 1H, CH_2), 4.76 (m, 1H, CH), 4.85 (m, 1H, CH), 5.64 (s, 1H, CH), 6.90 (m, 1H, NH), 7.06-7.22 (m, 5H, ArH), 7.45 (t, *J*=8 Hz, 1H, NH), 8.25 (s, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 24.2 (CH_3), 24.4 (CH_2), 25.1 (CH_2), 25.5 (CH_2), 33.2 (CH_3), 37.9 (CH_2), 48.1 (CH_2), 49.3 (CH_2), 52.2 (OCH_3), 53.1 (CH), 58.5 (C), 60.8 (CH), 63.6 (C), 67.9 (CH), 78.4 (CH), 114.4 (C), 126.8 (CH), 128.2 (CH), 128.4 (CH), 128.7 (CH), 129.1 (CH), 135.9 (C), 144.5 (C), 149.9 (C), 165.5 (CO), 168.4 (CO), 170.5 (CO), 171.9 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1681 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{26}H_{30}Br_2KN_6O_5S$ $[M+K]^+$: 734.99967; found 734.99935.

1.12. (2S)-Methyl 2-((2S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanamido)-4-methylpentanoate (7k).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.82-0.97 (m, 12 H, CH_3), 1.55 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.50-1.80 (m, 4H, CH_2), 1.63-1.81 (m, 2H, 2CH), 3.72 (s, 3H, OCH_3), 4.35 (dd, J_1= 13.7 Hz, J_2=10 Hz, 1H, CH_2), 4.49 (dd, J_1= 10 Hz, J_2= 3.9 Hz, 1H, CH), 4.60 (m, 1H, CH), 4.68-4.72 (m, 2H, CH and CH_2), 5.65 (s, 1H, CH), 6.80 (d, *J*= 8 Hz, 1H, NH), 7.49 (d, *J*= 8.4 Hz, 1H, NH), 8.32 (m, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 21.9 (CH_3), 22.0 (CH_3), 22.6 (CH_3), 22.8 (CH_3), 24.5 (CH_3), 24.7 (CH), 24.8 (CH), 33.2 (CH_3), 41.1 (CH_2), 41.4 (CH_2), 48.4 (CH_2), 50.7 (CH), 51.3 (CH), 52.4 (OCH_3), 58.5 (C), 63.7 (C), 67.8 (CH), 78.4 (CH), 126.2 (CH), 143.1 (C), 159.7 (CO), 165.6 (CO), 171.3 (CO), 173.3 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1651 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{24}H_{37}Br_2N_6O_5S$ $[M+H]^+$: 679.09074; found: 679.08736.

1.13. (2S)-Methyl 2-((2S,3S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (7l).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.92 (d, *J*= 6.2 Hz 3H, CH_3), 0.93 (d, *J*= 6.2, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.59-1.76 (m, 2H, CH_2), 1.61 (s, 3H, CH_3), 2.91-3.16 (m, 2H, CH_2), 3.76 (s, 3H, CH_3), 4.33 (m, 1H, CH_2), 4.51 (m, 1H, CH), 4.62 (m, 1H, CH), 4.80 (m, 1H, CH), 5.71 (s, 1H, CH), 6.44-6.85 (m, ArH), 6.76 (d, *J*= 8 Hz, 1H, NH), 7.37 (d, *J*= 8.4 Hz, 1H, NH), 8.22 (s, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 21.9 (CH_3), 22.9 (CH_3), 24.7 (CH_3), 33.2 (CH_3), 36.5 (CH_2), 39.9 (CH_2), 48.4 (CH_2), 51.1 (CH), 52.5 (CH), 53.0 (CH) 58.0 (C), 63.6 (C), 68.2 (CH), 78.5 (CH), 115.3 (CH), 126.9 (CH), 130.3 (CH), 142.8 (C), 155.0 (C), 159.8 (CO), 166.7 (CO), 170.8 (CO), 171.9 (CO). **IR:** (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1645 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{27}H_{35}Br_2N_6O_6S$ $[M+H]^+$: 729.0700; found: 729.06992.

1.14. (2S)-Methyl 2-((2S)-2-(1-(((2S,6S)-6-bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-(methylthio)butanamido)-3-phenylpropanoate (7m).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.58 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.00-2.23 (m, 2H, CH_2), 2.58 (t, *J*= 7 Hz, 2H, CH_2), 3.07 (dd, J_1= 13.8 Hz, J_2= 6.2 Hz, 1H, CH_2), 3.16 (dd, J_1= 14 Hz, J_2= 6.2 Hz, 1H, CH_2) 3.69 (s, 3H, OCH_3), 4.37 (dd, J_1= 14 Hz, J_2= 5.4 Hz, 1H, CH_2), 3.71 (s, 3H, OCH_3), 4.33-4.49 (m, 2H, CH and CH_2), 4.76-4.83 (m, 1H, CH), 4.85-4.92 (m, 1H, CH), 5.65 (s, 1H, CH), 6.78 (d, *J*= 8 Hz, 1H, NH), 7.07-7.22 (m, 5H, ArH), 7.67 (d, *J*= 8 Hz, 1H, NH), 8.19 (s, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 15.0 (CH_3), 24.5 (CH_3), 30.0 (CH_2), 31.1 (CH_2), 33.2 (CH_3), 37.8 (CH_2), 48.4 (CH_2), 51.6 (CH), 52.4 (OCH_3), 53.2 (CH), 58.5 (C), 63.6 (C), 67.9 (CH), 78.5 (CH), 126.0 (CH), 127.2 (CH), 128.6 (CH), 129.2 (CH), 135.5 (C), 143.1 (C), 159.6 (CO), 165.6 (CO), 170.1 (CO), 171.6 (CO). **IR:** (film) 1789 cm^{-1} (β-lactam), 1741 cm^{-1} (CO ester), 1651 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{26}H_{32}Br_2N_6NaO_5S_2$ $[M+Na]^+$: 753.0140; found: 753.01346.

1.15. (2S)-Methyl 2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanoate (7n).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.96 (s, 3H, CH_3), 0.98 (s, 3H, CH_3,), 1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.67-1.80 (m, 3H, CH and CH_2), 3.76 (s, 3H, OCH_3), 4.33-4.46 (m, 2H, CH and CH_2), 4.64-4.71 (m, 1H, CH_2), 4.78-4.86 (m, 1H, CH), 5.7 (s, 1H, CH), 7.46 (d, *J*= 8.6 Hz, 1H, NH), 8.35 (s, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 21.8 (CH_3), 22.8 (CH_3), 24.5 (CH), 24.8 (CH_3), 33.3 (CH_3), 41.5 (CH_2), 48.2 (CH_2), 50,5 (CH), 52.4 (OCH_3), 58.6(C), 63.5 (C), 67.9 (CH), 78.4 (CH), 126.4 (CH), 143.1 (C), 159.6 (CO), 165.6 (CO), 172.7 (CO). **IR:** (film) 1791 cm^{-1}(β-lactam), 1735 cm^{-1} (CO ester), 1649 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{18}H_{26}Br_2N_5O_4S$ $[M+H]^+$: 566.0072; found: 566.00711.

1.16. (2S)-Methyl 2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-3-(4-hydroxyphenyl)propanoate (7o).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.56 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 3.12 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.36 (m, 1H, CH_2), 4.38 (m, 1H), 4.65 (q, *J*= 11 Hz, 1H, CH_2), 5.01 (m, 1H, CH), 5.62 (s, 1H, CH), 6.69-7.04 (m, 4H, ArH), 7.53 (d, *J*= 8.6 Hz, 1H, NH), 8.09 (s, 1H, CH). **^{13}C NMR (CDCl$_3$, 75 MHz)** δ 24.5 (CH_3), 33.2 (CH_3), 37.6 (CH_2), 48.3 (CH_2), 52.4 (CH_3), 53.3 (CH), 58.4 (C), 63.6 (C), 67.9 (CH), 78.4 (CH), 115.5 (CH), 126.1 (CH), 127.4 (C), 130.4 (CH), 143.0 (C), 155.0 (C), 159.34 (CO), 165.5 (CO), 171.3 (CO). **IR:** (film) 1795 cm^{-1}(β-lactam), 1745 cm^{-1} (CO ester), 1655 cm^{-1} (CO amide). **HRMS**-ESI (*m/z)*: calcd for $C_{21}H_{23}Br_2N_5NaO_5S$ $[M+Na]^+$: 637.9684; found: 637.9678.

1.17. (2S)-Methyl 2-((2S)-2-(1-(((2S)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanamido)-3-phenylpropanoate (7p).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, *J*= 5.7 Hz, 3H, CH_3), 0.92 (d, *J*= 5.7 Hz, 3H, CH_3), 1.59 (s, 6H, 2 CH_3), 1.65-1.77 (m, 3H, CH and CH_2), 3.01-3.18 (m, 2H, CH_2), 3.06-3.13 (m , 1H, CH_2), 3.53 (dd, J_1= 16 Hz, J_2= 4.3 Hz, 1H, CH_2), 3.69 (s, 3H, OCH_3), 4.31-4.33 (m, 2H, CH and CH_2), 4.57-4.69 (m, 2H, CH and CH_2), 4.82-4.89 (m, 1H, CH), 5.16 (dd, J_1= 4 Hz, J_2= 2 Hz, 1H, CH), 6.73 (d, *J*= 7.6 Hz, 1H, NH), 7.05-7.11 (m, 5H, ArH), 7.35 (d, *J*= 8.3 Hz, 1H, NH), 8.19 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 21.8 (CH_3), 22.9 (CH_3), 24.6 (CH), 24.9 (CH_3), 33.0 (CH_3), 37.9 (CH_2), 40.5 (CH_2), 48.1 (CH_2), 49.07 (CH), 51.3 (CH), 52.3 (OCH_3), 53.2 (CH), 58.9 (CH), 63.7 (C), 68.1 (CH), 126.2 (CH), 127.0 (CH), 128.4 (CH), 129.2 (CH), 135.7 (C), 142.8 (C), 159.9 (CO), 171.0 (CO), 171.7 (CO), 173,2 (CO). **IR:** (film) 1776 cm^{-1} (β-lactam), 1745 cm^{-1} (CO ester), 1658 cm^{-1} (CO amide). **HRMS**-ESI (*m/z*): calcd for $C_{27}H_{37}N_6O_5S$ $[M+H]^+$: 557,2546; found: 557.25376.

1.18. (2S)-Methyl 2-((2S)-2-(1-(((2S)-6,6-dibromo-3,3-dimethyl-4,4-dioxido-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)methyl)-1*H*-1,2,3-triazole-4-carboxamido)-4-methylpentanamido)-3-phenylpropanoate (7q).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (d, *J*= 6 Hz, 3H, CH_3), 0.90 (d, *J*= 6 Hz, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.61-1.76 (m, 3H, CH and CH_2), 3.01-3.18 (m, 2H, CH_2), 3.05 (dd, J_1= 13.8 Hz, J_2= 6.3 Hz, 1H, CH_2), 3.13 (dd, J_1= 13.8 Hz, J_2= 5.9 Hz, 1H, CH_2), 3.68 (s, 3H, OCH_3), 3.52 (m, 1H, CH), 3.60-3.70 (m, 2H, CH_2), 4.70-4.78 (m, 1H, CH), 4.88 (m, 1H, CH), 5.16 (s, 1H, CH), 7.02 (d, *J*= 8.2 Hz, 1H, NH), 7.05-7.15 (m, 5H), 7.53 (d, *J*= 8.6 Hz, 1H, NH), 8.17 (s, 1H, CH). **^{13}C NMR ($CDCl_3$, 75 MHz)** δ 18.2 (CH_3), 19.8 (CH_3), 22.0 (CH_3), 22.8 (CH_3), 24.7 (CH), 37.9 (CH_2), 41.1 (CH_2), 44.8 (C), 49.1 (CH_2), 51.4 (CH), 52.4 (OCH_3), 53.3 (CH), 62.1 (CH), 64.5 (C), 74.0 (CH), 127.0 (CH), 127.3 (CH), 128.4 (CH), 129.2 (CH), 135.7 (C), 143.1 (C), 159.8 (CO), 165.2 (CO), 171.2 (CO), 171.9 (CO). **IR:** (film) 1807 cm^{-1} (β-lactam), 1745 cm^{-1} (CO ester), 1643 cm^{-1} (CO amide), 1336 and 1128 (sulfone). **HRMS**-ESI (*m/z*): calcd for $C_{27}H_{34}Br_2N_6NaO_7S$ $[M+Na]^+$: 767,0474; 7 found: 67.04602.

2. Anticancer screening

2.1 Cell lines and culture conditions

HeLa (ATCC CCL-2) cells were grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. B16-F0 cell line, kindly provided by the Laboratory of Molecular Oncology (Quilmes National University, Buenos Aires, Argentina), was grown in RPMI-1640 supplemented as indicated above. NMuMG (ATCC CRL-1636) cells, gently provided by the Institute of Oncology Angel H. Roffo (Buenos Aires, Argentina), were cultured in DMEM-F12 containing 10% FBS, 2 mM L-glutamine, 0.6% Hepes, 50 U/mL penicillin and 50 μg/mL streptomycin.

2.2 Cell proliferation assay

Cells were placed in 96-well microplates at a density of 2 x 10^4 cells/well (HeLa and NMuMG) or 1 x 10^4 cells/well (B16-F0) and incubated for 72 h at 37 °C in the presence or absence of 20 μM of the different compounds in a total volume of 0.2 ml of culture medium. Compounds were dissolved in dimethylsulfoxide (DMSO) as 10 mM stock solutions and stored at -70 °C. Prior to use, the compounds were diluted 1:10 in ethanol and added at the indicated concentrations to the culture medium. Control cells were treated under similar conditions and a final concentration of 20 μL vehicle/mL of culture medium was used in all experiments. Total cell number was evaluated by colorimetric determination of hexosaminidase levels, a ubiquitous lysosomal enzyme.[1] Briefly, cells were washed twice with phosphate-buffered saline and then incubated at 37 °C with 60 μL of 3.25 mM *p*-nitrophenol-*N*-acetyl-β-D-glucosaminide dissolved in 50 mM citrate buffer, pH 5, 0.25% Triton X-100. After 45-120 min, the color reaction was developed and the enzyme activity was blocked by adding 90 μL of 50 mM glycine buffer, pH 10.4, containing 5 mM EDTA. Absorbance values were measured at 405 nm in a visible plate reader (Biotrak II, AmershamBiosciences, USA). The molar drug concentrations required to cause 50% growth inhibition (IC_{50}) were determined from dose-response curves.

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Notes and references

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1 a) U. Landegren, *J. Immunol. Methods*, 1984, **67**, 379-388. b) M. Cárdenas, M. Marder, V.C. Blank, L.P. Roguin, *Bioorg. Med. Chem.*, 2006, **14**, 2966-2971.