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Analogues of SB-203207 as Inhibitors of tRNA Synthetases

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Abstract—SB-203207 and 10 analogues have been prepared, by elaboration of altemicidin, and evaluated as inhibitors of isoleucyl,. leucyl and valyl tRNA synthetases (IRS, LRS, and VRS, respectively). Substituting the isoleucine residue of SB-203207 with leucine and valine increased the potency of inhibition of LRS and VRS, respectively. The leucine derivative showed low level antibacterial activity, while several of the compounds inhibited IRS from *Staphylococcus aureus* WCUH29 more strongly than rat liver IRS.

Introduction

SB-203207 (**1**) was isolated from *Streptomyces* NCIMB 40513 and shown to inhibit isoleucyl tRNA synthetase (IRS) from *Staphylococcus aureus* Oxford and rat liver, with IC_{50} values of 1.7 and <2 nM, respectively.[1,2] The inhibition of IRS by SB-203207 (**1**) implies a structural resemblance between this compound and the enzyme's natural substrate. The natural substrates of other tRNA synthetases are the same as that of IRS, except that they have different amino acid side chains. Therefore, it seemed likely that replacing the isoleucine residue of SB-203207 (**1**) with, for example, leucine and valine would produce selective inhibitors of LRS and VRS, respectively. By analogy, Creppy et al.[3,4] reported that whereas ochratoxin A is an inhibitor of a phenylalanyl tRNA synthetase, substituting the phenylalanine residue of this compound with valine afforded a VRS inhibitor. To examine this hypothesis and carry out related structure–activity studies, SB-203207 (**1**) and the analogues **2**–**11** have been prepared from altemicidin (**12**), and evaluated as inhibitors of IRS, LRS, and VRS.

Synthesis

Compound **1** was not available from natural sources in sufficient quantity to allow for further testing or for use as a starting material in the preparation of analogues. Instead, we used altemicidin (**12**).[5] While this compound is available via total synthesis,[6] for our purposes it was produced through fermentation.[7] The methods used to synthesise compounds **1**–**3** and **6** from **12** are shown in Scheme 1.[8] Compound **10** corresponds to the intermediate **13** in the case when (*S*)-3-methylpentanoic acid was used as the acylating agent. Hydrogenolysis of **10** afforded **8**. Compound **5** was prepared using *t*Boc-(*S*)-isoleucine instead of the Cbz-derivative, and in that case the intermediate corresponding to **13** was deprotected through base hydrolysis, then acid hydrolysis. Compounds **4**, **7**, **9**, and **11** were prepared from altemicidin methyl ester[9] by applying similar methodology.

Inhibition Assays

The results of studies of the interactions of compounds **1**–**11** with IRS, LRS, and VRS are summarised in Table 1. The esters **4** and **5** are less potent than **1** as inhibitors of all the synthetases studied, and the des-amino derivative **6** of SB-203207 (**1**) is even less active. The more substantially modified analogues **7**–**11** show only low activity. SB-203207 (**1**) inhibits bacterial and mammalian IRS more strongly than LRS and VRS, and this behaviour is reflected with compounds **4**–**11**, presumably because each contains the *sec*-butyl side chain of isoleucine. In contrast, the leucine derivative **2** selectively inhibits LRS. The valine derivative **3** is more potent as an inhibitor of VRS than either SB-203207 (**1**) or the

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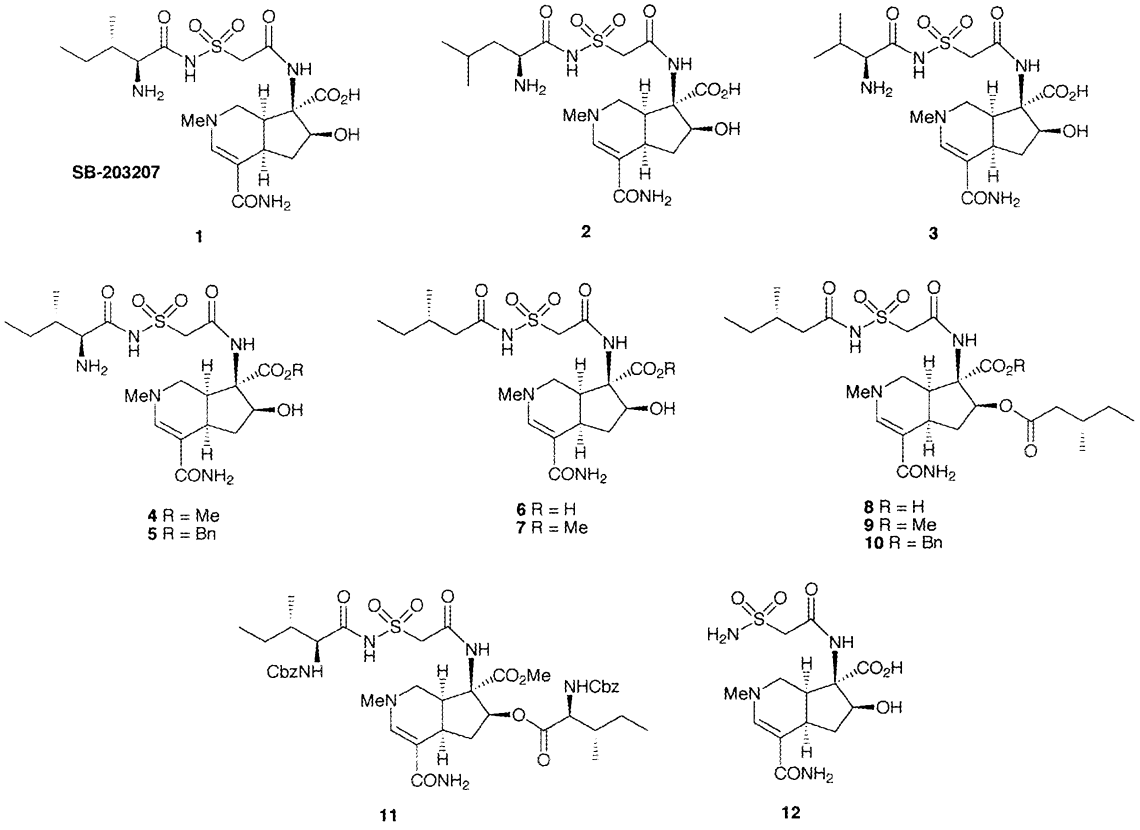
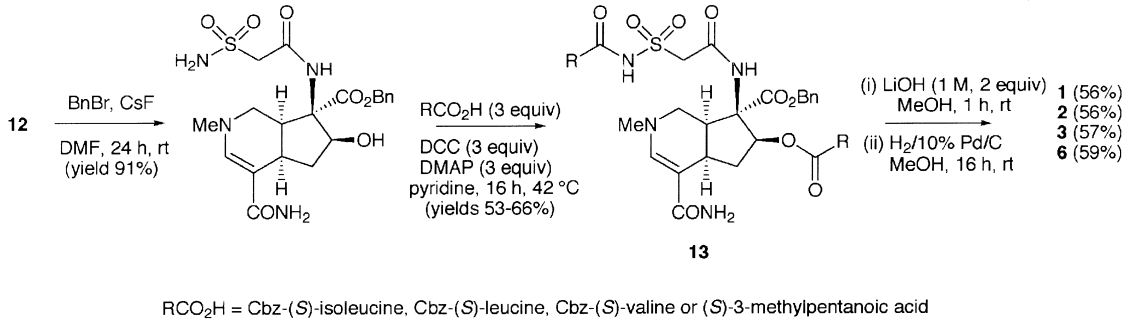


Table 1. Inhibition of tRNA synthetases by SB-203207 (**1**) and related compounds

Compound	Synthetase IC_{50} values (μM) or percentage inhibition[10]			
	IRS[a]	IRS[b]	LRS[a]	VRS[a]
1	0.014	0.0042	1.55	0.126
2	0.91	0.068	0.016	0.29
3	0.037	0.0029	2.3	0.03
4	0.06	0.017	186	40% @ 100 μM
5	0.12	0.025	NI @ 10 μM	NI @ 10 μM
6	3.27	22.3	NI @ 100 μM	NI @ 100 μM
7	23% @ 100 μM	25% @ 100 μM	13% @ 100 μM	NI @ 100 μM
8	14.6	6.1	NI @ 100 μM	65% @ 100 μM
9	18% @ 100 μM	9% @ 100 μM	NI @ 100 μM	NI @ 100 μM
10	19% @ 100 μM	20% @ 100 μM	NI @ 100 μM	6% @ 100 μM
11	46% @ 100 μM	0.5	NI @ 100 μM	NI @ 100 μM

[a]From *Staphylococcus aureus* WCUH29.
[b]From rat liver.



Scheme 1.

Table 2. Antibacterial activity of SB-203207 (**1**) and the leucine analogue **2**

Bacterial strain	MIC value (μg/mL)[11]	
	1	**2**
Staphylococcus aureus Oxford	>64	>64
Staphylococcus aureus WCUH29	>64	>64
Enterococcus faecalis 1	>64	>64
Enterococcus faecalis 7	>64	>64
Haemophilus influenzae Q1	64	64
Haemophilus influenzae NEMC1	64	64
Moraxella catarrhalis 1502	>64	>64
Streptococcus pneumoniae 1629	>64	32
Streptococcus pneumoniae N1387	>64	64
Streptococcus pneumoniae ERY2	64	16
Escherichia coli 7623 AcrABEFD+	>64	>64
Escherichia coli 120 AcrAB−	>64	>64

leucine analogue **2**. To this extent, the data for compounds **1**–**3** indicate that replacing the isoleucine moiety of SB-203207 (**1**) with other amino acid residues produces inhibitors of the corresponding synthetases. However, the leucine and valine derivatives **2** and **3** are also both potent inhibitors of IRS, more so than of VRS in the case of **3** and mammalian IRS. This lack of fidelity displayed by the synthetases is surprising.

Compounds **2**, **3**, and **11** show a marked preference for inhibition of the rat IRS over the bacterial protein, whereas compound **6** selectively binds to bacterial IRS. This indicates that there are differences between bacterial and mammalian tRNA synthetases that may allow development of antibiotics through selective inhibition of the bacterial enzymes. Compounds **3**–**11** did not show antibacterial activity against any of the organisms tested, but the leucine derivative **2** of SB-203207 (**1**) displayed low level activity (Table 2) and was more potent than **1** against three strains of *Streptococcus pneumoniae*.

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7. *Streptomyces* A5941 was grown according to the literature.[12] The extraction procedure at 300 L scale was as follows. The harvested broth was centrifuged and the supernatant was loaded onto a PK 208 cation exchange column. Altemicidin (**12**) was eluted with 0.5 M NH_4OH. The eluate was concentrated by reverse osmosis to low volume, and acidified to pH 2. The precipitate which formed was separated by centrifugation and discarded. The supernatant was loaded onto a Biotage HP2Oss cartridge at pH 2, the column was washed with water, and altemicidin (**12**) was eluted with 2% acetonitrile. The eluate was evaporated and freeze-dried. The product was further purified on a Sephadex G10 size exclusion column, then by crystallisation from water, to give pure altemicidin (**12**).
8. Data for compound **1**: mp 203–204 °C (decomp.); FTIR (KBr disk) 3440 (sh), 3357, 3229, 1718 (sh), 1633, 1510, 1393, 1282, 1114, 849 cm^{-1}; ^1H NMR (300 MHz, CD_3OD: 0.04 equiv AcOH) δ 0.96 (3H, t, J = 7.5 Hz), 1.05 (3H, d, J = 7.0 Hz), 1.17–1.33 (2H, m), 1.55–1.70 (1H, m), 1.94–2.09 (1H, m), 2.59–2.76 (1H, m), 2.81–3.05 (4H, m), 2.97 (3H, s), 3.57 (1H, d, J = 4.3 Hz), 4.10 (1H, J = 13.9 Hz), 4.30 (1H, J = 13.9 Hz), 4.24–4.34 (lH, m), 7.34 (1H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 12.1, 15.6, 25.5, 32.6, 38.0, 41.5, 43.1 (2 signals), 45.9, 58.5, 61.6, 67.9, 76.4, 99.5, 146.3, 165.9, 174.0, 175.5, 176.5; ESMS(−) 488 (M−H, 100); LSIMS-HRMS calcd for $C_{19}H_{32}N_5O_8S$ (M + H): 490.1973. Found: 490.1955. Anal. calcd for $C_{19}H_{31}N_5O_8S{\cdot}3H_2O$: C, 41.98; H, 6.86; N, 12.88. Found: C, 42.12; H, 6.69; N, 12.99%. Data for compound **2**: mp 195 °C (deform), 201 °C (melt); FTIR (KBr disk) 3435, 1717 (sh), 1634, 1510, 1384, 1340, 1282, 1112, 844 cm^{-1}; ^1H NMR (300 MHz, CD_3OD: 0.13 equiv AcOH) δ 0.97 (3H, d, J = 6.3 Hz), 0.99 (3H, d, J = 6.4 Hz), 1.27–1.32 (1H, m), 1.57–1.70 (1H, m), 1.74–1.87 (2H, m), 2.61–2.75 (1H, m), 2.82–3.04 (4H, m), 2.97 (3H, s), 3.66 (lH, dd, J = 5.0, 8.7 Hz), 4.12 (1H, J = 14.0 Hz), 4.30 (1H, J = 14.0 Hz), 4.24–4.36 (1H, m), 7.34 (1H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 22.4, 23.6, 25.9, 32.9, 41.7, 42.0, 43.5 (2 signals), 46.2, 55.9, 58.7, 68.2, 76.7, 99.8, 146.6, 166.2, 174.3, 176.9, 177.1; ESMS(−) 488 (M−H, 100); LSIMS-HRMS calcd for $C_{19}H_{32}N_5O_8S$ (M + H): 490.1973. Found: 490.1979. Anal. calcd for $C_{19}H_{31}N_5O_8S{\cdot}2H_2O$: C, 43.42; H, 6.71 N, 13.33. Found: C, 43.67; H, 6.47; N, l3.27%. Data for compound **3**: mp 199–202 °C (decomp.); FTIR (KBr disk) 3437, 1717 (sh), 1634, 1509, 1399, 1384, 1342, 1284, 1112, 851 cm^{-1}; ^1H NMR (300 MHz, CD_3OD: 0.12 equiv AcOH) δ 1.02 (3H, d, J = 6.9 Hz), 1.08 (3H, d, J = 7.0 Hz), 1.17–1.32 (1H, m), 2.36–2.41 (1H, m), 2.62–2.74 (1H, m), 2.78–3.03 (4H, m), 2.97 (3H, s), 3.51 (1H, d, J = 4.6 Hz), 4.12 (1H, J = 13.9 Hz), 4.31 (1H, J = 13.9 Hz), 4.24–4.36 (1H, m), 7.34 (1H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 17.8, 19.6, 31.4, 32.9, 41.8, 43.4 (2 signals), 46.2, 58.8, 62.7, 68.2, 76.7, 99.8, 146.6, 166.2, 174.3, 176.0, 176.9; ESMS(−) 474 (M−H, 100); LSIMS-HRMS calcd for $C_{18}H_{30}N_5O_8S$ (M + H): 476.1815. Found: 476.1824. Anal. calcd for $C_{18}H_{29}N_5O_8S{\cdot}1.5H_2O$: C, 43.02; H, 6.42; N, 13.94. Found: C, 42.88; H, 6.39; N, 13.77%. Data for compound **6**: mp 165.5–168.0 °C; FTIR (KBr disk) 3457 (sh), 3358, 3237 (sh), 1712, 1670 (sh), 1638, 1507, 1463, 1404, 1342, 1290, 1157, 1143, 1080, 906, 865 cm^{-1}; ^1H NMR (300 MHz, CD_3OD: 0.15 equiv AcOH) δ 0.91 (3H, t, J = 7.5 Hz), 0.95 (3H, d, J = 6.7 Hz), 1.16–1.32 (2H, m), 1.34–1.47 (1H, m), 1.84–1.97 (1H, m), 2.13 (1H, dd, J = 8.1, 14.8 Hz), 2.35 (1H, dd, J = 6.3, 14.8 Hz), 2.60–2.72 (1H, m), 2.80–3.02 (4H, m), 2.96 (3H, s), 4.25–4.30 (1H, m), 4.42 (1H, J = 14.5 Hz), 4.56 (1H, J = 14.5 Hz), 7.34 (1H, s); ^{13}C NMR (75 MHz, CD_3OD) δ 11.6, 19.4, 30.3, 32.5, 33.0, 41.4, 43.l, 43.2, 44.4, 45.8, 57.9, 67.9, 76.2, 99.6, 146.2, 163.5, 174.0, 175.0, 176.1; ESMS(−) 473 (M−H, 100); LSIMS-HRMS calcd for $C_{19}H_{31}N_4O_8S$ (M + H): 475.1864. Found: 475.1843. Anal. calcd for $C_{19}H_{30}N_4O_8S{\cdot}1.5H_2O$: C, 45.50; H, 6.63; N, 11.17. Found: C, 45.82; H, 6.52; N, 11.15%.

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11. Whole-cell antibacterial activity was determined by broth microdilution. Compounds were dissolved in DMSO and diluted 1:10 in water to produce a 256 μg mL^{-1} stock solution. Using a 96-well microtitre plate, a Microlab AT Plus 2 (Hamilton Co., Reno, NV) serially diluted 50 μL of the stock solution into supplemented cation adjusted Mueller Hinton broth (Beckton Dickinson, Cockeysville, MD). After the compounds were diluted, a 50 μL aliquot of the test isolate ($\sim 1 \times 10^6$ cfu mL^{-1}) was added to each well of the microtitre plate using the Microlab AT Plus 2. The final test concentrations ranged from 0.06 to 64 μg mL^{-1}. Inoculated plates were incubated at 35 °C in ambient air for 18–24 h. Following incubation, a microtitre mirror reader (Cooke Instruments Ltd, UK) was used to assist in reading the minimum inhibitory concentration (MIC). The MIC was determined as the lowest concentration of compound that inhibited visible growth of the test isolates.
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