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Discovery of a Biologically Active Thiostrepton Fragment

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Thiostrepton (**1**, Figure 1) and its biological activities have attracted considerable attention from the scientific and medical communities. Among thiostrepton's biological properties, those against bacteria,[1] tumor cells,[2] and *Plasmodium falciparum*,[3] the parasite responsible for human malaria, are the most prominent; immunosuppressive properties have also been reported.[4] Originally isolated from *Streptomyces azureus* ATCC 14921,[5] and subsequently from *Streptomyces hawaiiensis* ATCC12236 and *Streptomyces laurentii* ATCC 31255, this thiopeptide antibiotic[6] has recently succumbed to total synthesis in these laboratories.[7] It was during this research program that we had the opportunity to design and synthesize an array of fragments of thiostrepton representing its various regions and structural motifs. In this communication, we wish to report on the discovery of a biologically active thiostrepton fragment that, despite its relatively small size, retains, and in some instances surpasses, the biological properties of the natural substance.

Although the antibacterial action of thiostrepton was attributed to its binding to the 23S region of the bacterial ribosomal RNA and protein L11, thereby inhibiting the GTPase-dependent function of the 50S ribosomal RNA and thus inhibiting protein biosynthesis,[8] knowledge regarding the precise nature of the binding and structure activity relationships within this area is lacking. Our synthetic schemes developed for the total synthesis of thiostrepton allowed us to access the four regions of the molecule (**2**−**5**), representing the dehydropiperidine core (**2**), the bis-dehydroalanine tail (**3**), the thiazoline−thiazole moiety (**4**), and the quinaldic acid subunit (**5**), as shown in Figure 1. With the support of this enabling technology, we initiated a program directed toward the construction of these fragments and certain of their derivatives for biological screening purposes, hoping to uncover a smaller pharmacaphore within the structure of thiostrepton. Thus, starting with the previously synthesized substrates **2a**−**5a**,[7a,b] compounds **2**−**5** were prepared in one step in each case as shown in Figure 2 [**2a** → **2**, $PdCl_2(PPh_3)_2$ (cat.)-*n*-Bu_3SnH[9] (100% yield); **3a** → **3**, TBAF (90% yield); **4a** → **4**, HF•py (78% yield); **5a** → **5**, HF•py (70% yield)].

Given the intriguing architecture of the dehydropiperidine core **2**, we also chose to prepare a number of its derivatives (**6**, **8**) and its 5*S*,6*R* isomer (**7**), as shown in Figure 3. Thus, exposure of **2** to pentafluorophenol acetate (PFPA)[10] afforded selectively, and in 73% yield, the bis-acetate **6**. On the other hand, the imine functionality within the *N*-Alloc-protected dehydropiperidine **2a** was stereoselectively[11] reduced by the action of $NaCNBH_3$ and AcOH to afford the secondary amine **8a** (67% yield), from which the *N*-Alloc group was removed by treatment with $PdCl_2(PPh_3)_2$ (cat.)-*n*-Bu_3SnH,[9] furnishing compound **8** in 85% yield. Finally, the 5*S*,6*R* diastereomer **7**[7b,d] of the dehydropiperidine core **2** was generated from its *N*-Alloc derivative **7a** by the action of $PdCl_2(PPh_3)_2$ (cat.)-*n*-Bu_3SnH in quantitative yield.

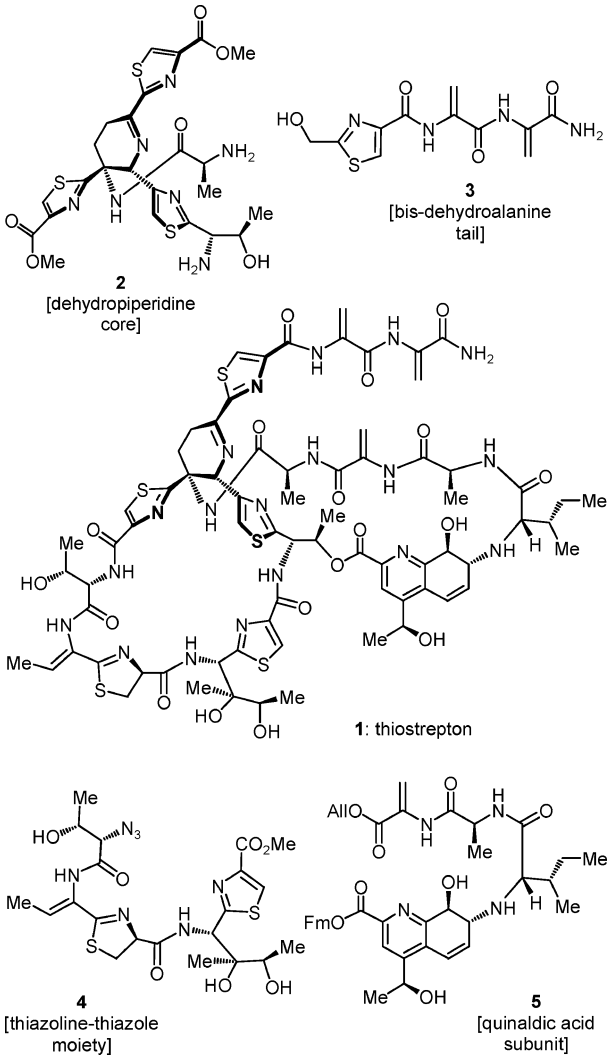

Figure 1. Molecular structures of thiostrepton (**1**) and fragments (**2**−**5**) representing its four domains.

The synthesized compounds (**2**−**8**), together with thiostrepton, vancomycin, and teicoplanin, were tested for their activity against the two Gram-positive bacterial strains, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VREF), as well as against *Escherichia coli* (EC), a Gram-negative strain. As shown in Table 1, the dehydropiperidine core compounds **2** and **8** were found to possess significant activity against MRSA, while they were proven to be not active (NA) against EC, with the derivative with the imine functionality intact (**2**) being the more potent of the two. The 5.0 μM MIC value of **2** against MRSA is indeed impressive, given its molecular simplicity

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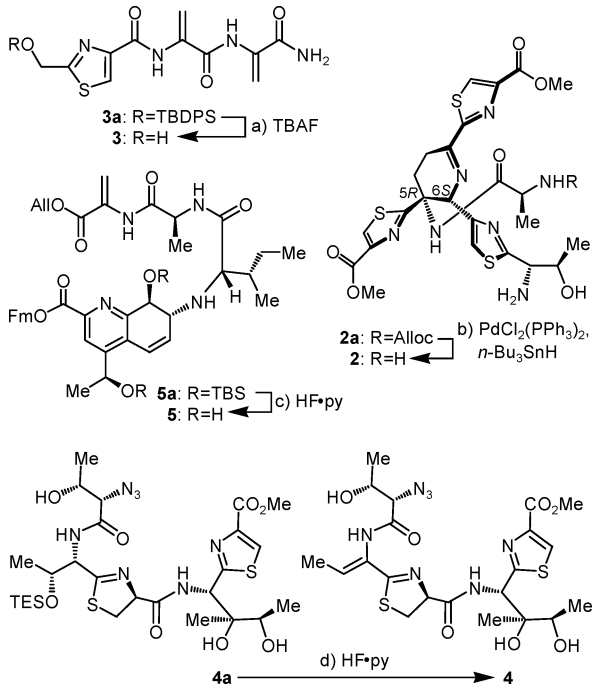

Figure 2. Synthesis of thiostrepton fragments **2**−**5**. Reagents and conditions: (a) TBAF (1.2 equiv), THF, 0 °C, 30 min, 90%; (b) $PdCl_2(PPh_3)_2$ (0.1 equiv), *n*-Bu_3SnH (50 equiv), CH_2Cl_2, −45 °C, 10 min, then 0 °C, 30 min, 100%; (c) HF•py:THF (1:4), 0 °C, 5 min, then 25 °C, 24 h, 70%; (d) HF•py (1:4), THF, 0 °C, 5 min, then 25 °C, 18 h, 78%. TBDPS = *tert*-butyl diphenylsilyl; TBAF = tetra-*n*-butylammonium fluoride; Alloc = allyloxy carbonyl; Ph = phenyl; TBS = *tert*-butyl dimethylsilyl; Fm = fluorenylmethyl; All = allyl; py = pyridine.

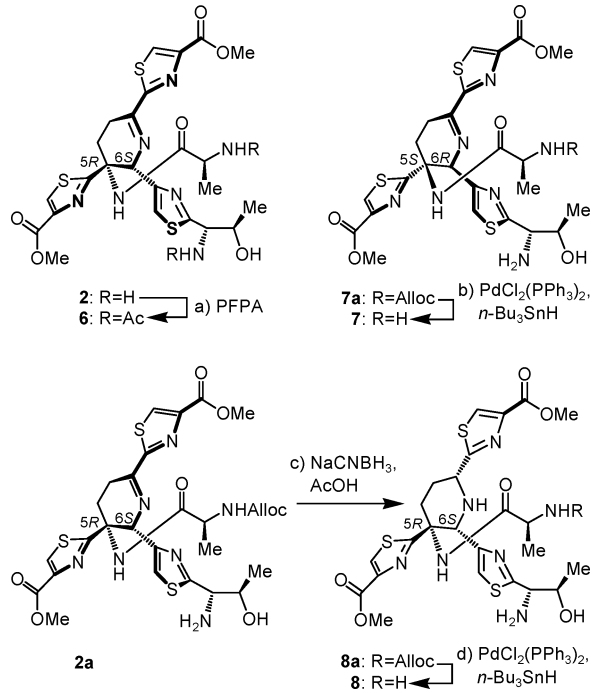

Figure 3. Synthesis of thiostrepton fragments **6**−**8**. Reagents and conditions: (a) pentafluorophenol acetate (5.0 equiv), DMF, 25 °C, 18 h, 73%; (b) $PdCl_2(PPh_3)_2$ (0.1 equiv), *n*-Bu_3SnH (50 equiv), CH_2Cl_2, −45 °C, 10 min, then 0 °C, 30 min, 100%; (c) $NaCNBH_3$ (4.0 equiv), EtOH:AcOH (4:1), 0 °C, 1 h, 67%; (d) $PdCl_2(PPh_3)_2$ (0.1 equiv), *n*-Bu_3SnH (3.4 equiv), CH_2Cl_2, 0 °C, 30 min, 85%. PFPA = pentafluorophenol acetate; AcOH = acetic acid.

compared to that of thiostrepton (**1**), being only 20 times less than that for thiostrepton, 10 times less than that for teicoplanin, and only 5 times less than that observed for vancomycin (see Table 1). Furthermore, compound **2** exhibited the same activity against VREF, being only 5 times less potent than thiostrepton in that test (Table 1). Importantly, the dehydropiperidine core compound **2** showed a pronounced ability to differentiate between human and bacterial cells, as the concentration required to hemolyze 50% of a sample of human red blood cells (HD_{50}) was found to be 150 μM (Table 1). This 30-fold difference in antibacterial and hemolytic activity may provide a useful therapeutic window for a potential drug candidate, should one be pursued. It is noteworthy that two earlier studies focusing on the thiazoline−thiazole domain[13a] and the bis-dehydroalanine side chain[13b] of thiostrepton failed to produce any compounds exhibiting significant in vitro antibacterial activity. In contrast, the present investigation, expanded to include all four regions of thiostrepton, expeditiously resulted in the discovery of a relatively potent antibacterial lead, which by virtue of its low molecular weight as compared to the natural product may lead to an improved pharmacological profile.

Table 1. In Vitro Antibacterial and Hemolytic Activities of Thiostrepton (**1**) and its Synthetic Fragments (**2**−**8**)[12]

	MIC (μM)[a]			
compound	MRSA[b]	VREF[c]	EC[d]	HD_{50}[e] (μM)
vancomycin	1.0	NA[f]	NA	ND[g]
teicoplanin	0.5	ND	NA	ND
thiostrepton (**1**)	0.2	1.0	NA	>200
2	5.0	5.0	NA	150
3	NA	NA	NA	ND
4	NA	NA	NA	>200
5	NA	NA	NA	>200
6	NA	NA	NA	ND
7	NA	NA	NA	ND
8	15.0	ND	NA	>200

[a] Minimum inhibitory concentration. [b] Methicillin-resistant *Staphylococcus aureus* ATCC 33591. [c] Vancomycin-resistant *Enterococcus faecalis* ATCC 51575. [d] *Escherichia coli* ATCC 29425. [e] Concentration required to hemolyze 50% of human red blood cells. [f] NA: not active at the highest concentration tested (50 μM). [g] ND: not determined.

Compound **2** may or may not exert its antibacterial activity through the same mechanism of action as thiostrepton, and further studies will be needed to answer that question. However, the fact that bis-acetate derivative **6** does not exert any activity against these bacterial strains, while the triamine **8** does retain some activity, suggests that an aminoglycoside-like mechanism of action[14] may be operating whereby dehydropiperidine compound **2** binds to a specific RNA site. That **2** binds to a specific site, rather than a random binding pocket, is also corroborated by the inability of its 5*S*,6*R* diastereomer (i.e., compound **7**) to mimic its biological action.

Encouraged by these results and the single report attributing antitumor activity to thiostrepton,[2] we proceeded to test the synthesized compounds (**2**−**8**) against a set of tumor cell lines, including human non-small cell lung cancer (NCI-H460), human colon cancer (HCT-116), human ovarian cancer (SK-OV-3), human breast cancer (MCF-7), and human chronic myelogenous leukemia cancer (K-562) cell lines. Shown in Table 2, the results were interesting, for dehydropiperidine compound **2** exhibited higher potency than thiostrepton against all cell lines tested (average LC_{50} = 0.9 μM for **2** and 2.3 μM for thiostrepton; see Table 2). The reduced compound **8** was also active against the same cell lines with an average LC_{50} value of 2.0 μM (see Table 2). Significantly, the quinaldic acid subunit **4**, which was devoid of any antibacterial action, exhibited considerable activity against these tumor cells (LC_{50} = 8.8 μM average value; see Table 2).

Prompted by the observation of the promising cytotoxic properties of compound **2**, we then proceeded to test its action against

Table 2. In Vitro Anticancer Activities of Thiostrepton (**1**) and its Synthetic Fragments (**2**−**8**)[12]

	LC_{50} (μM)[a]				
compound	NCI-H460[b]	HCT-116[c]	SK-OV-3[d]	MCF-7[e]	K-562[f]
doxorubicin	0.042	0.12	0.075	0.36	0.08
Taxol	0.043	0.017	0.042	0.018	0.021
thiostrepton (**1**)	1.5	1.6	2.8	3.8	1.7
2	0.9	0.6	1.2	0.9	0.8
3	NA[g]	NA	NA	NA	NA
4	5.7	7.3	8.0	17.0	6.0
5	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA
8	1.9	1.6	2.7	2.0	1.7

[a] Concentration required to kill 50% of the cell. [b] Human nonsmall cell lung cancer cell line. [c] Human colon cancer cell line. [d] Human ovarian cancer cell line. [e] Human breast cancer cell line. [f] Human chronic myelogenous leukemia cancer cell line. [g] NA: not active at the highest concentration tested (50 μM).

Table 3. In Vitro Activities of Thiostrepton (**1**) and its Synthetic Fragment **2** against Ovarian Cancer Cell Lines[a]

	IC_{50} (μM)[b]			
compound	1A9[c]	PTX10[d]	A8[e]	AD10[f]
epothilone A	0.0024	0.0051	0.041	0.029
epothilone B	0.0010	0.0078	0.010	0.025
Taxol	0.0015	0.032	0.0008	>0.15
thiostrepton (**1**)	0.96	1.1	0.9	91.0
2	0.07	0.1	0.2	0.4

[a] Cells were plated in 96-well plates and incubated with the test compounds for 72 h. The cells were then fixed and processed for growth using the sulforhodamine B assay.[15] [b] Concentration required to inhibit 50% of cell proliferation. [c] Parental ovarian cancer cell line. [d] Taxol-resistant ovarian cancer cell line. [e] Epothilone A-resistant ovarian cancer cell line. [f] P-glycoprotein (pgp)-overexpressing ovarian cancer cell line.

the human ovarian cancer cell line 1A9 and its drug-resistant mutants PTX10 (taxol-resistant), A8 (epothilone A-resistant), and AD10 (pgp-overexpressing). As shown in Table 3, this dehydropiperidine fragment (**2**) exhibited remarkable potency against all these cell lines (average $IC_{50} = 0.19$ μM; see Table 3), being significantly more potent than its parent compound, thiostrepton (**1**) (average $IC_{50} = 23$ μM; see Table 3).

Combining significant antibacterial and antitumor activity, compound **2** represents an important finding in that it provides a new lead for further exploration in chemical biology studies and drug discovery efforts. The discovery of **2** also demonstrates the potential benefits of total synthesis endeavors and the power of complex molecule construction in biology and medicine. Further studies to improve the potency of compound **2** and to elucidate its mode of action are warranted.

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Supporting Information Available: Experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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