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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5366–5369

Aaptamines as sortase A inhibitors from the tropical sponge *Aaptos aaptos*

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Received 7 May 2007; revised 12 July 2007; accepted 3 August 2007
Available online 11 August 2007

Abstract—Four aaptamines (**1**–**4**), 1*H*-benzo[*de*][1,6]-naphthyridine alkaloids, were isolated from the marine sponge *Aaptos aaptos* and their inhibitory activities against sortase A (SrtA), an enzyme that plays a key role in cell wall protein anchoring and virulence in *Staphylococcus aureus*, were evaluated. Isoaaptamine (**2**) was a potent inhibitor of SrtA, with an IC_{50} value of 3.7 ± 0.2 μg/mL. The suppression of fibronectin-binding activity by isoaaptamine (**2**) highlights its potential for the treatment of *S. aureus* infections via inhibition of SrtA activity. Our studies have identified a series of SrtA inhibitors, providing the basis for further development of potent inhibitors.

Many surface proteins of Gram-positive bacteria are covalently anchored to bacterial cell wall peptidoglycans through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases known as sortases.[1] These surface proteins play pivotal roles in the adhesion to specific organ tissues, invasion of host cells, and the evasion of host-immune responses.[2,3] Sortase A (SrtA) has been identified in *Staphylococcus aureus* and shown to be required for cell wall anchoring of protein A and virulence in this bacterium.[4–7] *S. aureus* mutants lacking sortase fail to display surface proteins and are defective in the establishment of infections.[8] Because many of the known surface proteins of Gram-positive bacteria are believed to be exported and anchored via the sortase pathway, inhibitors of SrtA may be promising candidates for the treatment and/or prevention of Gram-positive bacterial infections.

Marine sponges are proving to be productive sources of many interesting biologically active nitrogen-containing heterocyclic compounds, including a series of 1*H*-benzo[*de*][1,6]-naphthyridine alkaloids,[9–11] and the aaptamines have been shown to form a small group of 1*H*-benzo[*de*][1,6]-naphthyridine alkaloids. The parent naphthyridine, known as aaptamine, was first isolated by Nakamura and coworkers[9] from the marine sponge *Aaptos aaptos* and was found to possess antineoplastic activity and cancer cell growth inhibitory activity.[12] Isoaaptamine was first reported from a sponge in the genus *Suberites*[13] and was later also isolated from *A. aaptos*.[14,15] This compound has been reported to be a PKC inhibitor[16] and to inhibit growth of cancer cells.[14,15] Demethylaaptamine and demethyloxyaaptamine from the Okinawan and Taiwanese marine sponge *A. aaptos* has also shown significant cytotoxicity against human tumor cells.[10,15] Recently, Pettit and coworkers described the synthetic conversion of aaptamine to isoaaptamine and dimethylaaptamine and reported the antimicrobial activity of these compounds against clinically important pathogenic bacteria and yeasts.[17,18]

Our group has been interested in finding biologically active secondary metabolites from marine sponges. During our continuing program, we encountered the marine sponge *A. aaptos* from the Federated States of

Keywords: Aaptamines; Sponge; *Aaptos aaptos*; *Staphylococcus aureus*; Sortase A inhibition.
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doi:10.1016/j.bmcl.2007.08.007

Micronesia, and a crude extract from the sponge exhibited significant inhibitory activity against *S. aureus* SrtA. Bioassay-guided separation of the crude extract using various chromatographic techniques yielded four aaptamines, which were potent SrtA inhibitors. We report the isolation and biological activities of these compounds.

The specimens of *A. aaptos* were collected by hand using SCUBA at a depth of 15–20 m from Chuuk Atoll, Federated States of Micronesia, in August 2005.[19] Frozen sponge was lyophilized (dry weight 0.42 kg), macerated, and repeatedly extracted with MeOH (1 L × 3) and CH_2Cl_2 (1 L × 2). The extract was filtered and concentrated under reduced pressure to afford 25.4 g of crude extract (IC_{50} = 56.8 μg/mL). The residue was partitioned between *n*-BuOH and H_2O. A portion (400 mg) of the *n*-BuOH layer (5.05 g, IC_{50} = 35.4 μg/mL) was subjected to LH-20 gel-permeation chromatography using 100% MeOH as eluent and yielded four distinct fractions (F3–F6). F3 and F4 were dried (IC_{50} = 20.5 μg/mL) and separated by C_{18} reversed phase HPLC (YMC ODS-A column, 1 × 25 cm, 80% MeCN containing 0.3% TFA) to yield compounds **1**–**3**. F5 and F6 also were dried (IC_{50} = 30.2 μg/mL) and separated by reversed phase HPLC (with 40% aqueous MeOH) to yield compound **4**. The purified compounds **1**–**4** were isolated in the following amounts: 70, 20, 20, and 4 mg, respectively.

Based on the results of combined spectral analyses and comparison of spectral data with those of known compounds, the compounds were identified as aaptamine (**1**),[9] isoaaptamine (**2**),[13–15] and demethylaaptamine (**3**).[17] The minor compound was also identified as demethyloxyaaptamine (**4**).[14,15] Spectral data for these compounds were consistent with those reported previously (Fig. 1).

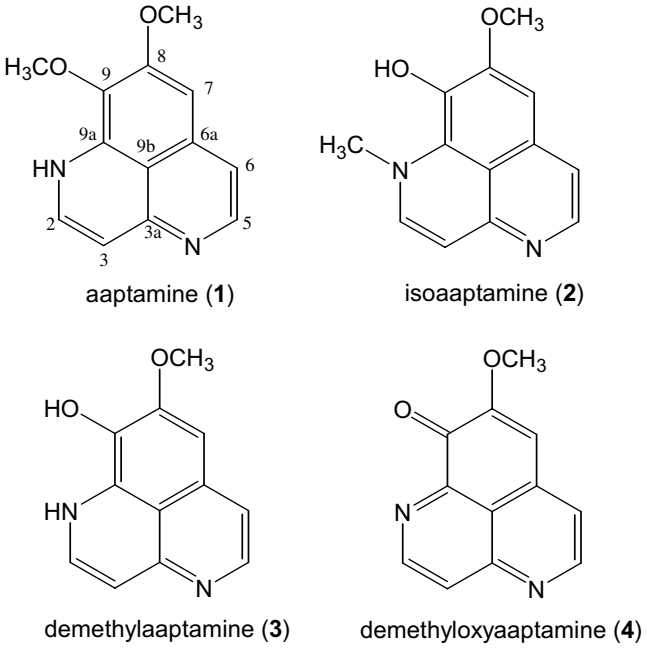

Figure 1. Structures of aaptamines.

Compounds **1**–**4** were evaluated for inhibitory activity against *S. aureus* SrtA according to a previously published procedure.[20,21] The inhibitory potencies, expressed as IC_{50} values, of the tested compounds are shown in Table 1 and are compared to that of a known SrtA inhibitor, berberine chloride (IC_{50}: 8.7 ± 0.3 μg/mL).[22] Compound **2** exhibited the most potent inhibitory activity against SrtA (IC_{50}: 3.7 ± 0.2 μg/mL). Compounds **1**, **3**, and **4** were less effective than berberine chloride, with IC_{50} values of 23.5 ± 0.8, 17.2 ± 0.6, and 20.1 ± 0.7 μg/mL, respectively. The SrtA inhibitory activity study of these compounds revealed that the methyl group at the N-1 position of compound **2** was important (Fig. 1). Compounds **1**, **3**, and **4,** which are unmethylated at the N-1 position, were less active than **2** (Table 1). Methylation and oxidation of the hydroxyl group at the C-9 position of **3,** as in compounds **1** and **4**, respectively, exhibited almost the same inhibitory activity against SrtA as compound **3**. These results suggest that the SrtA inhibitory activities of the aaptamines are altered by substitution at the N-1 position.

It is well-known that sortase inhibitors should act as anti-infective agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability.[8,23] To rule out effects of the test compounds on *S. aureus* (strain Newman)[24] cell adhesion to fibronectin due to inhibition of cell growth, the minimum inhibitory concentration (MIC) of compounds **1**–**4** were determined by the microtiter broth dilution method.[25] The four compounds (**1**–**4**) isolated from *A. aaptos* exhibited no or moderate growth inhibitory activity against *S. aureus* strain Newman, with MIC values of >200, 50, 25, and 25 μg/mL, respectively (Table 1). Based on the MIC test data, compound **2** (MIC: 50 μg/mL) was selected and used in the fibronectin-binding assay.

An active sortase enzyme is required for the attachment of *S. aureus* to eukaryotic cell matrices. Mutant *S. aureus* strains lacking a functional sortase cannot bind to cell matrix proteins, such as fibrinogen and fibronectin, and also cannot effect protein A-mediated binding of IgG in vitro.[3] We postulated that sortase inhibitors would inhibit SrtA activity in vivo and in turn reduce fibronectin-binding protein surface display. Thus, we employed an assay[25,26] in which cell adhesion to fibronectin-coated plates was quantified by measuring the absorbance following staining with crystal violet. The capacities of *S. aureus* strain Newman ($srtA^+$) and its isogenic knockout mutant SKM12 ($srtA^-$)[24] in adhering

Table 1. Inhibitory effects of aaptamines on the activity of SrtA enzyme and bacterial growth of *S. aureus* strain Newman

Compound	SrtA IC_{50}[a] (μg/mL)	MIC (μg/mL)
1	23.5 ± 0.8	>200
2	3.7 ± 0.2	50
3	17.2 ± 0.6	25
4	20.1 ± 0.7	25
Berberine chloride	8.7 ± 0.3	100

[a] IC_{50} values are means ± SD (n = 3). Berberine chloride was used as a reference inhibitor of SrtA.

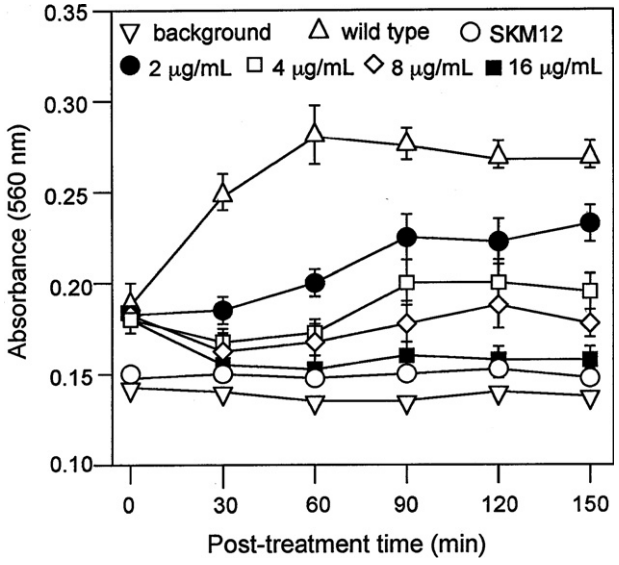

Figure 2. Inhibition of *S. aureus* adhesion to fibronectin by isoaaptamine (**2**). Adhesion of wild-type Newman (*srtA*$^{+}$) (untreated), SKM12 (*srtA*$^{-}$) (untreated), and wild-type Newman, treated with 2, 4, 8, and 16 μg/mL of isoaaptamine (**2**). The values are means ± SD (n = 3).

to fibronectin-coated surfaces were investigated. As shown in Figure 2, the fibronectin-binding activity of SKM12 significantly decreased compared to that of the wild type (Newman). These results are consistent with those previously observed with the wild-type and isogenic knockout mutant.[8] It has also been reported that SKM12 displayed a 2-log reduction in virulence (>99% reduction of bacteria in kidneys) compared with the wild-type strain (Newman) in a mouse model infection test.[8] A second area of interest regarding the SrtA inhibitor isoaaptamine (**2**) was its fibronectin-binding inhibitory properties. As expected, treatment of strain Newman with isoaaptamine (**2**) reduced the capacity of the bacterium to adhere to fibronectin-coated surfaces in a dose-dependent manner (0–16 μg/mL, Fig. 2). The onset and magnitude of the inhibition of fibronectin-binding in *S. aureus* treated with isoaaptamine (**2**) (>16 μg/mL) was comparable to the behavior of untreated SKM12.

In conclusion, we isolated aaptamines as SrtA inhibitors of *S. aureus* from the marine sponge *A. aaptos* based on a bioassay. The methyl group at the N-1 position of isoaaptamine proved to be an important factor for SrtA activity, changing selectivities and IC_{50}s, when comparing the structures of the isolated aaptamines. The fibronectin-binding activity data highlight the potential of these compounds in the treatment of *S. aureus* infections via inhibition of sortase activity.

Acknowledgments

We are grateful to the Department of Marine Resources, State of Chuuk, Federated States of Micronesia for allowing marine organisms research. This study was supported by Grant R01-2004-000-10429-0 from the Basic Research Program of the Korea Science & Engineering Foundation. This research was partially supported by the KORDI (PE97802), the Ministry of Maritime Affairs and Fisheries (PM44700), Korea Research Foundation Grant (KRF-2004-005-F00055), and the Brain Korea 21 project.

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DMSO), which was found to have no effect on enzyme activity at concentrations of less than 1%. Appropriate blanks contained all of the above, with the exception of the test sample. Reactions were carried out for 1 h at 37 °C and analyzed fluorometrically at 350 nm for excitation and 495 nm for recordings.

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