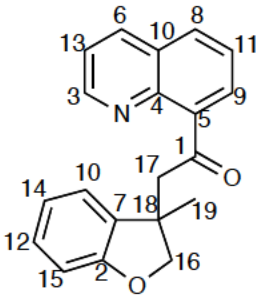
Smithtolidins A and F: Isolation, Structure Determination, and Biological Activity

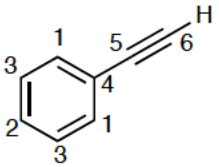
K[redacted] and K[redacted]

Department of Chemistry, University of Minnesota, Minneapolis, MN 55455-0431

Abstract



Smithtolidin A



Smithtolidin F

Smithtolidin A and F are two new interesting molecules that have been isolated from the sponge *Cabineta nuclear* from the depths of Smith sea located in the tundra known as Minnesota. We report the isolation, characterization and the unique biological properties of these molecules. It has been found smithtolidin A relieves stress and anxiety in human health, most noticeably in graduate students while its doppelganger, smithtolidin F, promotes procrastination, also most active in graduate student cell lines and most noticeably when in combination with smithtolidin A.

Smithtolidin A and F have been recently investigated targets of interest due to their unique biological activities that have previously been unobserved. They have been isolated together from the sponge *Cabineta nuclear,* deep in Smith sea, in the now frozen tundra of Minnesota. In cell lines GS-06, smithtolidin A was found to promote success while simultaneously relieving stress and anxiety. Smithtolidin F was found to produce the opposite effects in these cell lines, promoting procrastination and sluggish or lazy behavior (Table 1).

Table 1. Success and Failure Assay Results with GS-06 Cells

	GI_{50} (μM)
smithtolidin A	0.085 ± 0.003
smithtolidin F	0.093 ± 0.008

When combined with smithtolidin A, the effects of A dominate and are enhanced in the presence of the smithtolidin F, known as its doppelganger. The unique properties and symbiotic activities of these molecules have motivated further investigation and characterization.

Smithtolidin F was characterized using MS, IR and NMR methods. The molecular

weight was found to be 102, indicating a formula of C_8H_6. The infrared spectrum revealed a terminal alkyne (3291 and 2110 cm^{-1}) and an aromatic ring (1674 and 1488 cm^{-1}). The overtones from 1700 to 2000 cm^{-1} indicated a monosubstituted aromatic ring. The structure was confirmed by 2 *sp* and 6 sp^2 carbons in the ^{13}C spectrum, as well as corresponding protons in the ^{1}H spectrum (Table 2). DEPT and COSY experiments provided additional verification of the structure.

Table 2. ^{1}H and ^{13}C NMR Data for Smithtolidin **F**[a]

no.	™$_C$	δ_H (*J* in Hz)
1	132.2	7.55 - 7.52 (m)
2	128.8	7.39 - 7.33 (m)
3	128.3	7.39 - 7.33 (m)
4	122.1	
5	83.7	
6	77.3	3.10 (s)

[a] $CDCl_3$ 300 MHz for ^{1}H, 75 MHz for ^{13}C, δ in ppm.

Smithtolidin A was characterized using Hi-Res MS, IR and various NMR methods including ^{1}H, ^{13}C, COSY, HMQC and HMBC. The mass spectrum revealed an $[M + Na]^+$ peak of 326, and therefore a molecular weight of 303 g/mol and a hydrogen deficiency index of 10 were determined. Using this information along with functional group indicators in the IR and NMR spectra, a molecular formula of $C_{20}H_{17}NO_2$ was obtained.

By inspection of the aromatic regions of the ^{1}H and ^{13}C spectra (Table 3), compound A was found to contain a quinoline substructure. Further analysis of coupling in the COSY and HMBC spectra indicated substitution at the 8 position of the ring system. An additional aromatic ring was required elsewhere in the compound to accommodate the remaining aromatic protons and carbons. The COSY and HMBC coupling of these protons allowed for further elucidation, giving a fused benzene and dihydrofuran ring system.

Table 3. ^{1}H and ^{13}C NMR Data for Smithtolidin **A**[a]

no.	™$_C$	δ_H (*J* in Hz)
1	204.7	
2	159.1	
3	150.5	8.94 (dd, 1.8, 4.2)
4	145.5	
5	140.1	
6	136.3	8.19 (dd, 1.7, 8.3)
7	135.5	
8	131.2	7.92 (dd, 1.3, 8.2)
9	128.9	7.81 (dd, 1.4, 7.1)
10	128.2	
10'	128.2	7.12 - 7.09 (m)
11	126.1	7.56 (dd, 8.0, 8.0)
12	122.9	7.12 - 7.09 (m)
13	121.5	7.45 (dd, 4.2, 8.3)
14	120.3	6.83 - 6.79 (m)
15	109.7	6.83 - 6.79 (m)
16	83.0	4.71 (d, 9.2); 4.58 (d, 9.2)
17	54.2	3.95 (d, 17.6); 3.8 (d, 17.6)
18	44.4	
19	25.9	1.56 (s)

[a] $CDCl_3$ 500 MHz for ^{1}H, 125 MHz for ^{13}C, δ in ppm.

A key IR peak found at 1681 cm^{-1} revealed a ketone functionality. Analysis of the HMBC spectra showed the carbonyl carbon to be connected directly to the quinoline system. The opposite side of the ketone was found to be methylene carbon 17 by similar multiple bond coupling analysis.

Finally, a tertiary methyl group was observed at 1.56 ppm in the ^{1}H spectrum. At this point all that remained was to connect the quinoline, furan and methyl fragments. This could essentially be done in two ways: the quinoline and methyl structures attaching to separate tertiary carbons on the furan, or both fragments attaching to the

same quaternary carbon. Simple proton coupling seen in the COSY spectrum required the quaternary carbon, the specific location of which was determined by the proton and carbon shifts of the furan ring. Methylene carbon 16 was found to be adjacent to the oxygen, while the quaternary center is directly connected to the aromatic ring. This final structure was further corroborated by the remaining COSY and HMBC data.

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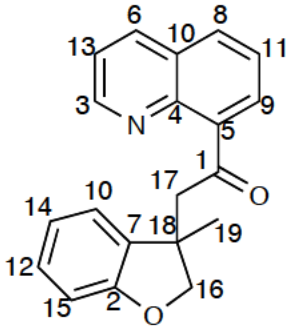
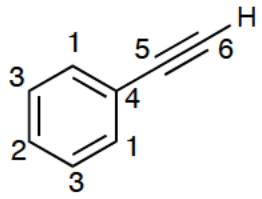
Supporting Information Available: Free of charge at the end of this document.

Supporting Information:



Smithtolidin A: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.94 (dd, J = 1.8, 4.2 Hz, 1 H), 8.19 (dd, J = 1.7, 8.3 Hz, 1 H), 7.92 (dd, J = 1.3, 8.2 Hz, 1 H), 7.81 (dd, J = 1.4, 7.1 Hz, 1 H), 7.56 (dd, J = 8.0, 8.0 Hz, 1 H), 7.45 (dd, J = 4.2, 8.3 Hz, 1 H), 7.12-7.09 (m, 2 H), 6.83-6.79 (m, 2 H), 4.71 (d, J = 9.2, 1 H), 4.58 (d, J = 9.2, 1 H), 3.95 (d, J = 17.6, 1 H), 3.80 (d, J = 17.6, 1 H), 1.56 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.7, 159.1, 150.5, 145.5, 140.1, 136.3, 135.5, 131.2, 128.9, 128.2, 128.2, 126.1, 122.9, 121.5, 120.3, 109.7, 83.0, 54.2, 44.4, 25.9; IR (thin film) 3046, 2961, 2881, 1681, 1595, 1568, 1475, 971, 831, 793, 751, 1136, 1056, cm^{-1}; GC-MS m/z (%relative intensity): 326.1208 $(M + Na)^+$ (100), 304.1337 $(M + H)^+$ (11), 172.0754 (53).



Smithtolidin F: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55-7.52 (m, 2 H), 7.39-7.33 (m, 3 H), 3.10 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.2, 128.8, 128.3, 122.1, 83.7, 77.3; IR (thin film) 3291, 3081, 3058, 3034, 2110, 1954, 1900, 1886, 1808, 1757, 1488, 757, 692, 666, 621, 530 cm^{-1}; GC-MS m/z (%relative intensity): 102 (M^+) (100), 76 (20), 63 (9), 50 (5), 39 (3).