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ALKALOIDS FROM THE MARINE ISOLATE OF THE FUNGUS *Aspergillus fumigatus*

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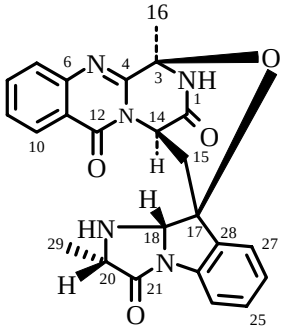
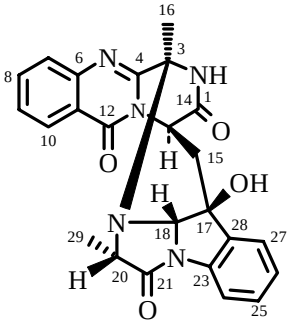
We previously isolated from the marine isolate of the fungus *Aspergillus fumigatus* KMM 4631 the five diketopiperazine antibiotics cyclotryprostatins A and B, fumitremorgin C, 12, 13-dihydrofumitremorgin C, and verruculogen [1]. Further research on metabolites of this strain afforded **1**-**5**.

Cultivation was performed under the previously described conditions [1]. The $CHCl_3$:C_2H_5OH extract of *A. fumigatus* was chromatographed over a column of silica gel L (40/100 μm) with elution successively by $CHCl_3$ and $CHCl_3$:C_2H_5OH (10:1 and 5:1). Fractions eluted by $CHCl_3$:C_2H_5OH (10:1) were combined, concentrated in vacuum to the minimal volume, and separated by HPLC over columns of Silasorb Si (ethylacetate:hexane, 4:1) and Diasphere-110-C18 (from 50 to 60% CH_3OH in water) to afford **1** (4 mg), **2** (18 mg), **3** (3 mg), **4** (3.5 mg), and **5** (4 mg).



1



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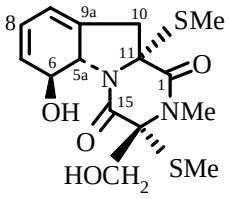

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Fumiquinazoline C (1), mp 239-243°C (ethylacetate), $C_{24}H_{21}N_5O_4$, $[\alpha]_D^{22}$ -160.4° (*c* 0.027, $CHCl_3$), R_f 0.70 (silica-gel plates, Sorbfil, Sorbpolymer, Russia, ethylacetate:hexane, 6:1). FABMS, *m*/*z*: 444 (37) $[M + H]^+$, 426 (7), 373 (5), 256 (6), 228 (26), 217 (12), 199 (41), 185 (45), 171 (18), 146 (20), 93 (100).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 170.8 (C-1), 84.2 (C-3), 150.4 (C-4), 146.3 (C-6), 128.4 (C-7), 134.9 (C-8), 128.6 (C-9), 127.0 (C-10), 121.4 (C-11), 159.5 (C-12), 51.5 (C-14), 31.5 (C-15), 24.6 (C-16), 87.2 (C-17), 87.0 (C-18), 58.6 (C-20), 170.0 (C-21), 135.8 (C-23), 115.4 (C-24), 130.2 (C-25), 126.2 (C-26), 124.8 (C-27), 138.3 (C-28), 18.7 (C-29).

Fumiquinazoline D (2), mp 210-214°C (ethylacetate), $C_{24}H_{21}N_5O_4$, mp 212-215°C (ethylacetate), $[\alpha]_D^{22}$ +86.2° (*c* 0.15, $CHCl_3$), R_f 0.45. FABMS, *m*/*z*: 444 (31) $[M + H]^+$, 426 (20), 315 (6), 256 (6), 228 (40), 199 (100).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 172.4 (C-1), 70.9 (C-3), 152.3 (C-4), 146.4 (C-6), 127.8 (C-7), 134.9 (C-8), 127.7 (C-9), 126.9 (C-10), 120.5 (C-11), 161.0 (C-12), 52.8 (C-14), 43.5 (C-15), 19.0 (C-16), 84.1 (C-17), 85.7 (C-18), 59.2 (C-20), 171.5 (C-21), 137.6 (C-23), 115.5 (C-24), 130.1 (C-25), 125.8 (C-26), 124.3 (C-27), 137.6 (C-28), 17.5 (C-29).

Spectral data and two-dimensional spectra (DEPT, COSY-45, HSQC, HBMC, and NOESY) of **1** and **2** agree with those in the literature for fumiquinazolines C and D isolated from the marine isolate of *A. fumigatus* [2].

Fumitremorgin B (3), $C_{27}H_{33}N_3O_5$, $[\alpha]_D^{22}$ +16.4° (*c* 0.3, $CHCl_3$), R_f 0.70. Mass spectrum (EI, 70 eV, *m*/*z*): 479 (71) $[M]^+$, 462 (8), 311 (100), 268 (51), 240 (17), 214 (22), 200 (16), 187 (9), 70 (12).

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^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 131.0 (C-2), 104.3 (C-3), 121.2 (C-4), 104.2 (C-5), 156.0 (C-6), 93.9 (C-7), 137.8 (C-8), 120.4 (C-9), 69.0 (C-10), 82.9 (C-11), 170.2 (C-12), 49.0 (C-14), 22.6 (C-15), 28.9 (C-16), 58.7 (C-17), 166.0 (C-18), 58.4 (C-20), 122.9 (C-21), 134.9 (C-22), 25.7 (C-23), 18.4 (C-24), 45.2 (C-25), 120.2 (C-26), 134.6 (C-27), 25.5 (C-28), 18.2 (C-29), 55.7 (C-30).

Spectral data for **3** agree with those in the literature for fumitremorgin B isolated from *A. fumigatus* parasitic on rice [3, 4].

Bisdethiobis(methylthio)gliotoxin (4), $C_{15}H_{20}N_2O_4S_2$, $[\alpha]_D^{20}$ -46.4° (*c* 0.04, CH_3OH), R_f 0.52. Mass spectrum (EI, 70 eV, *m*/*z*): 356 (8) $[M]^+$, 309 (51) $[M - SMe]^+$, 279 (26), 261 (100), 231 (73).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.88 (1H, m, H-5$_a$), 4.92 (1H, m, H-6), 5.88 (1H, m, H-7), 5.73 (1H, m, H-8), 5.93 (1H, m, H-9), 2.90, 2.96, 3.05, 3.10 (2H, dd, J = 16.0, H-10), 2.24 (3H, s, 12S–CH_3), 3.15 (3H, s, 13N–CH_3), 2.27 (3H, s, 14S–CH_3), 3.88, 3.92, 4.37, 4.41 (2H, dd, J = 12.0, H-15).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 165.9 (C-1), 71.9 (C-3), 166.8 (C-4), 69.6 (C-5$_a$), 74.4 (C-6), 123.1 (C-7), 130.1 (C-8), 120.0 (C-9), 131.6 (C-9$_a$), 38.9 (C-10), 71.5 (C-11), 14.6 (C-12), 28.7 (C-13), 13.7 (C-14), 63.6 (C-15). HBMC correlations (H/C): H-5$_a$/C-9$_a$, C-9; H-6/C-5$_a$, C-7, C-8; H-7/C-5$_a$, C-6; H-8/C-9; H-9/C-9$_a$; H-10/C-5$_a$, C-9$_a$, C-9, C-11; 12S–CH_3/C-11; 13N–CH_3/C-1, C-3; 14S–CH_3/C-3; H-15/C-3, C-4.

Mass spectra and PMR spectra of **4** agree with those reported previously for bisdethiobis(methylthio)gliotoxin isolated from the fungi *Gliocladium deliquescens* [5], *A. fumigatus* [6], and *Penicillium terlikowskii* [7].

Tryptoquivaline J (5), $C_{22}H_{18}N_4O_4$, mp 250-255°C (acetone), $[\alpha]_D^{22}$ +59.0° (*c* 0.3, $CHCl_3$), R_f 0.48. FABMS, *m*/*z*: 403 (100) $[M + H]^+$, 239 (32), 211 (16), 199 (68), 147 (27).

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 5.75 (1H, s, H-2), 7.67 (1H, br.d, J = 7.5, H-5), 7.25 (1H, t, J = 7.5, H-6), 7.40 (1H, dd, J = 3.3, 7.7, H-7), 7.82 (1H, d, J = 7.7, H-8), 6.35 (1H, t, J = 11, H-12), 2.85 (1H, dd, J = 3.4, 12.7, H-13$_a$), 3.05 (1H, t, J = 12.7, H-13$_b$), 4.62 (1H, q, J = 6.9, H-15), 8.48 (1H, br.d, J = 7.8, H-20), 7.43 (1H, dd, J = 3.1, 7.8, H-21), 7.70 (1H, dd, J = 3.1, 8.1, H-22), 7.88 (1H, d, J = 8.1, H-23), 1.82 (3H, d, J = 7, CH_3-15).

^{13}C NMR spectrum (75.4 MHz, C_5D_5N): 8.40 (C-2), 77.2 (C-3), 137.0 (C-4), 125.0 (C-5), 125.8 (C-6), 130.2 (C-7), 115.3 (C-8), 139.7 (C-9), 166.2 (C-11), 55.9 (C-12), 39.7 (C-13), 172.4 (C-14), 60.0 (C-15), 160.9 (C-18), 122.5 (C-19), 127.0 (C-20), 127.1 (C-21), 134.3 (C-22), 127.7 (C-23), 148.3 (C-24), 148.9 (C-26), 14.4 (15-CH_3). HMBC correlations (H/C): H-2/C-3, C-9; H-5/C-3, C-7, C-9; H-6/C-4, C-8; H-7/C-5, C-9; H-8/C-4, C-6; H-12/C-11, C-13, C-18; 2H-13/C-2, C-3, C-11, C-12; H-15/C-14, 15-CH_3; CH_3-15/C-14, C-15; H-20/C-18, C-22, C-24; H-21/C-19, C-23; H-22/C-20, C-24; H-23/C-19, C-21; H-26/C-18, C-24.

Mass spectra and PMR spectra of **5** agree with those reported previously in the literature for tryptoquivaline J isolated from the fungus *A. fumigatus* parasitic on rice [8].

Chemical shifts in the PMR spectra of **4** and **5** in the literature were not assigned for all protons. Chemical shifts of the C atoms have not been reported. Shifts of protons and C atoms in **4** and **5** were assigned using DEPT, COSY-45, HSQC, and HBMC spectra.

Thus, *A. fumigatus* KMM 4631 is a new source of alkaloids of various structural types. Bisdethiobis(methylthio)gliotoxin (**4**) and tryptoquivaline J (**5**) were isolated from marine isolates of fungi for the first time.

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