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New Indolocarbazoles from a Mutant Strain of the Marine-Derived Actinomycete *Streptomyces fradiae* 007M135

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ABSTRACT

Fradcarbazoles A−C (1−3), three new indolocarbazoles, were isolated from a mutant strain of the marine-derived actinomycete *Streptomyces fradiae* 007M135. Their structures were established by spectroscopic analysis, quantum chemical calculation, CD spectra, and chemical transformation. Fradcarbazole A (1) possessed a unique skeleton consisting of a staurosporine core, a thiazole ring, and an indole fragment. Compounds 1−3 displayed significant cytotoxicity against HL-60, K562, A-549, and BEL-7402 cell lines and inhibitory effects on the kinase PKC-α with IC_{50} values of 0.001−4.58 μM.

Since the isolation of the first indolocarbazole (ICZ) staurosporine in 1977,[1] more than 130 indolocarbazoles (ICZs) have been isolated from different organisms including bacteria, fungi, and invertebrates.[2] This family of compounds has evolved into an interesting class and has attracted great attention for their unusual structures and important biological activities,[3] such as cytotoxicity,[4] protein kinase C (PKC),[5] and topoisomerase (Topo)[6] inhibitions. Although staurosporine has not been used in antitumor applications because of its low selectivity and high toxicity, its analogues, such as UCN-01 and PKC412, are currently being tested in the cancer clinical trials.[7] As part of our ongoing research on new antitumor ICZs from marine-derived actinomycetes,[8] *Streptomyces fradiae* 007 was isolated and identified from a sediment sample collected in Jiaozhou Bay, Shandong Province of China. The EtOAc extracts of the fermentation broth of strain 007

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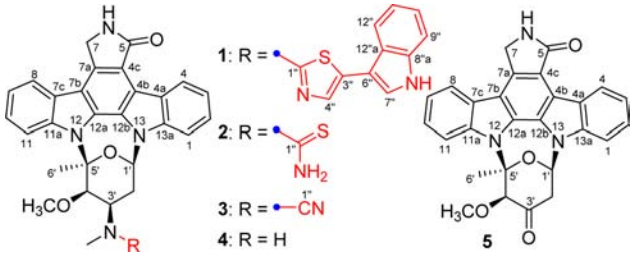
exhibited a significant cytotoxic effect on the P388 cell line and were detected to contain a large number of staurosporine and its analogues by HPLC-UV-MS. In order to enlarge the production of staurosporine and obtain structurally new ICZ analogues, a mutant strain, *S. fradiae* 007M315, was obtained from *S. fradiae* 007 after a compound mutation using ultraviolet and nitrosoguanidine.[9] The titer of strain M315 to produce staurosporine was nine times as that of the original strain 007.[9] Chemical investigation on the EtOAc extract of the fermentation broth (350 L) of strain M315 resulted in the isolation and identification of three new ICZs, which we have named fradcarbazoles A−C (**1**−**3**, 3.9, 6.0, and 3.0 mg, respectively), as well as seven known analogues, staurosporine (**4**, 2.2 g),[10] 3′-demethylamino-3′-oxostaurosporine (**5**, 8.0 mg),[11] 3′-*N*-aminoformylstaurosporine (**6**, 16.0 mg),[12] 3′-*N*-formylstaurosporine (9.0 mg),[13] 3′-*N*-acetylstaurosporine (3.2 mg),[14] K252a (4.2 mg),[15] and K252c (20.0 mg).[16] Fradcarbazole A possessed a unique skeleton consisting of a staurosporine core, a thiazole ring, and an indole fragment. This multiheterocyclic skeleton was very infrequent in the family of ICZs and was found in nature for the first time.



Fradcarbazole A (**1**) gave an HRESIMS peak at *m*/*z* 665.2354 $[M + H]^+$, corresponding to the molecular formula $C_{39}H_{32}N_6O_3S$.[17] Its UV spectrum showed a characteristic peak of ICZ chromophore at λ_{max} 224, 283, 325, and 364 nm.[8] Careful comparison of its ^1H and ^{13}C NMR spectra (Table 1) with those of staurosporine indicated a staurosporine unit that was confirmed by $^1H-^1H$ COSY of H-1/H-2/H-3/H-4, H-8/H-9/H-10/H-11, and H-1′/H-2′/H-3′/H-4′ and the key HMBC correlations of H-4 to C-13a and C-4b, H-6 to C-4c and C-7a, H-8 to C-7b and C-11a, H-11 to C-7c, H-1′ to C-12b and C-5′, H-6′ and 4′-OCH_3 to C-4′, and 3′-NCH_3 to C-3′ (Figure 1). In addition to the signals of the staurosporine unit, signals of six olefinic methine carbons and five olefinic quaternary carbons were also observed. $^1H-^1H$ COSY of H-9″/H-10″/H-11″/H-12″ and the key HMBC correlations of H-8″ to C-6″ and C-12″a; H-7″ to C-6″, C-12″a, and C-8″a; and H-12″ to C-8″a suggested an indole moiety. The rest of C_3HNS consisted of a thiazole nucleus from the HMBC correlations of H-4″ to C-1″ and C-3″. These structural fragments were confirmed by the Q-TOF MS^2 peaks at *m*/*z* 230, 286, 328, 354, and 436 (Figures 3 and S24). The key HMBC correlations of 3′-NCH_3 to C-1″ and H-7″ to C-3″ suggested that the thiazole moiety further connected staurosporine and indole moieties into fradcarbazole A (**1**) via two single bonds between 3′-N and C-1″ and between C-3″ and C-6″ (Figure 1). This connectivity was further confirmed by ^{13}C NMR quantum mechanical calculations of model compounds (Figure S1 and Table S1). The relative configuration of **1** was elucidated by NOESY experiment which showed correlations of H-1′ to H-3 and H-6′ to H-4′ and H-3′ (Figure 1), suggesting the same relative configuration to staurosporine. Compound **1** displayed similar CD Cotton effects at 296 ($\Delta\varepsilon$ +11.7), 248 ($\Delta\varepsilon$ +6.3), and 208 ($\Delta\varepsilon$ −10.4) nm to those of staurosporine at 299 ($\Delta\varepsilon$ +6.3), 249 ($\Delta\varepsilon$ +4.5), and 206 ($\Delta\varepsilon$ −14.9) nm (Figure 2), indicating the same absolute configuration of **1** to staurosporine. Moreover, an extension of the lactone sector rule[8a,18] was used to confirm the deduction. The molecule was viewed from the line on the plane of the amide group along the bisectrix of the N—C═O angle, *i.e.*, the line from C-5 to C-1′ as shown in Figure 3 for (1′*R*, 3′*R*, 4′*R*, 5′*S*)-**1**. The functional group at C-5′ lying in the back upper right sector was responsible for the positive Cotton effect ($\pi-\pi^*$ transition of conjugated lactam) that was good in accordance with the measured CD Cotton effect at λ_{max} 297 nm of **1** (Figure 2). Thus, the absolute configuration of **1** was unambiguously established to be (1′*R*, 3′*R*, 4′*R*, 5′*S*)-. Accordingly, the structure of **1** was identified as 3′-*N*-(5-(1*H*-indol-3-yl)-thioazol-2-yl)staurosporine.

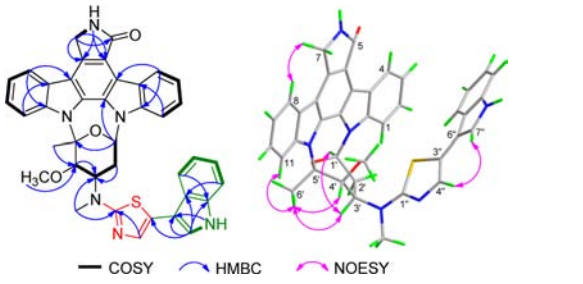


Figure 1. $^1H-^1H$ COSY, HMBC, and NOESY correlations of **1**.

The molecular formula of fradcarbazole B (**2**) was assigned to be $C_{29}H_{27}N_5O_3S$ based on the HRESIMS.[19]

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(17) Fradcarbazole A (**1**): yellow, amorphous powder, $[\alpha]_D^{21}$ +31 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 224 (3.26), 283 (3.59), 325 (3.15), 364 (3.08) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 296 (+11.7), 261 (+0.6), 248 (+6.3), 241 (+4.5), 231 (+5.3), 208 (−10.4) nm; IR (KBr) ν_{max}: 2924, 1677, 1586, 1533, 1467, 1342, 1315, 1118, 743 cm^{-1}; HRESIMS *m*/*z* 665.2354 $[M + H]^+$ (calcd for $C_{39}H_{33}N_6O_3S$, 665.2335).
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Table 1. ^{1}H and ^{13}C NMR Data for Fradcarbazoles A–C (**1**–**3**)[a]

	1[b]		**2**		**3**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	108.9, CH	7.68, d (8.3)	109.7, CH	7.71, d (8.2)	107.5, CH	7.20, d (8.1)
2	125.2, CH	7.49, ddd (8.3, 7.4, 1.1)	125.8, CH	7.48, ddd (8.3, 7.1, 1.2)	125.7, CH	7.49, dd (8.1, 7.2)
3	119.5, CH	7.31, dd (7.9, 7.3)	120.0, CH	7.29, ddd (7.9, 7.1, 1.0)	120.4, CH	7.38, dd (7.9, 7.2)
4	125.6, CH	9.31, d (8.0)	126.2, CH	9.28, d (7.9)	126.9, CH	9.42, d (7.9)
4a	122.6, C		123.2, C		123.6, C	
4b	115.2, C		115.8, C		116.5, C	
4c	119.4, C		119.9, C		119.1, C	
5	171.8, C		172.5, C		173.1, C	
6		8.61, brs		8.61, brs		6.56, brs
7	45.3, CH_2	5.00, s	45.9, CH_2	4.99, s	45.9, CH_2	5.00, d (17.9); 4.97, d (17.9)
7a	132.5, C		133.2, C		132.8, C	
7b	114.1, C		114.7, C		114.6, C	
7c	123.7, C		124.4, C		124.9, C	
8	121.4, CH	8.07, d (7.8)	122.0, CH	8.05, d (7.7)	121.5, CH	7.92, d (7.7)
9	120.2, CH	7.37, dd (7.8, 7.4)	120.9, CH	7.35, dd (7.7, 7.1)	120.8, CH	7.37, dd (7.7, 7.2)
10	124.9, CH	7.48, ddd (8.6, 7.4, 1.1)	125.6, CH	7.48, ddd (8.2, 7.1, 1.0)	125.1, CH	7.47, dd (8.3, 7.2)
11	113.4, CH	8.03, d (8.6)	114.3, CH	8.01, d (8.2)	112.4, CH	7.77, d (8.4)
11a	138.7, C		139.4, C		138.6, C	
12a	129.2, C		129.7, C		130.4, C	
12b	125.1, C		125.9, C		126.3, C	
13a	136.2, C		136.8, C		136.7, C	
1′	82.3, CH	7.10, dd (8.6, 6.6)	82.9, CH	7.06, dd (8.3, 7.1)	81.9, CH	6.66, dd (8,2, 7.0)
2′	27.0, CH_2	2.88, ddd (13.1, 8.4, 3.9); 2.40, ddd (13.1, 13.1, 6.5)	28.1, CH_2	2.69, ddd (13.1, 8.3, 3.7); 2.21, ddd (13.1, 13.1, 6.7)	29.4, CH_2	2.84, ddd (13.5, 8.3, 4.4); 2.73, ddd (13.5, 11.3, 6.8)
3′	53.3, CH	4.98, ddd (13.2, 3.9, 2.9)	54.2, CH	4.00, ddd (13.1, 3.6, 2.1)	55.6, CH	3.65, ddd (11.1, 4.5, 2.3)
4′	82.5, CH	4.51, d (2.8)	82.9, CH	4.45, d (2.1)	83.2, CH	4.04, d (2.2)
5′	94.7, C		95.5, C		93.5, C	
6′	29.1, CH_3	2.44, s	29.9, CH_3	2.38, s	29.0, CH_3	2.43, s
3′-NCH_3	34.4, CH_3	2.92, s	33.2, CH_3	2.78, s	37.6, CH_3	2.76, s
4′-OCH_3	60.1, CH_3	2.73, s	60.8, CH_3	2.73, s	60.3, CH_3	2.66, s
1″	167.4, C		182.6, C		116.5, C	

[a] ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz for **1** in DMSO-d_6, 600 and 150 MHz for **2** in DMSO-d_6, and 500 and 125 MHz for **3** in $CDCl_3$, respectively. [b] The data for $\delta_{C\text{-}3''}$–$\delta_{C\text{-}12''a}$ and $\delta_{H\text{-}4''}$–$\delta_{H\text{-}12''}$ were 120.4 (C, C-3″), 133.5 (CH, C-4″), 106.8 (C, C-6″), 122.7 (CH, C-7″), 136.4 (C, C-8″a), 111.8 (CH, C-9″), 121.7 (CH, C-10″), 119.4 (CH, C-11″), 119.0 (CH, C-12″), and 124.8 (C, C-12″a), and 7.56 (s, H-4″), 7.58 (d, J = 2.0 Hz, H-7″), 11.35 (d, J = 1.9 Hz, H-8″), 7.44 (d, J = 8.1 Hz, H-9″), 7.18 (ddd, J = 8.1, 7.8, 1.0 Hz, H-10″), 7.12 (ddd, J = 7.9, 7.8, 1.0 Hz, H-11″), and 7.82 (d, J = 7.9 Hz, H-12″), respectively.

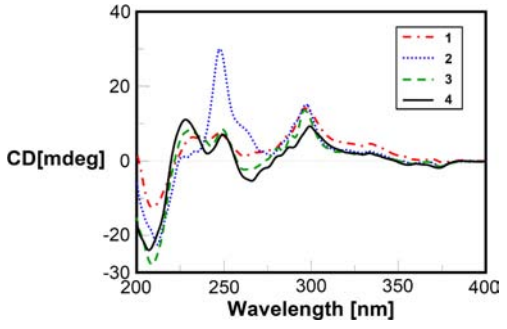


Figure 2. CD spectra of compounds **1**–**3** and staurosporine (**4**).

The ^{1}H and ^{13}C NMR data of **2** (Table 1) were very similar to those of staurosporine[10] except for an additional carbon signal (δ_C 182.6) corresponding to a thiourea. Combined with the molecular formula and the HMBC correlation between 3′-NCH_3 and C-1″ (Figure S2), the structure of **2** was identified to be a thioaminoformyl substituted derivative of staurosporine at position 3′-N. The NOESY spectrum of **2** also showed a similar correlation pattern to that of **1** (Figure S2), indicating the same relative configuration. The CD Cotton effects at 297 ($\Delta\varepsilon$ +9.8), 248 ($\Delta\varepsilon$ +19.7), and 212 ($\Delta\varepsilon$ −15.0) nm (Figure 2) suggested the same absolute configuration to **1**. Thus, compound **2** was elucidated as 3′-*N*-thioaminoformylstaurosporine.

The molecular formula for fradcarbazole C (**3**) was assigned to be $C_{29}H_{25}N_5O_3$ from the HRESIMS peak at m/z 492.2044 $[M + H]^+$.[20] Compared with those of staurosporine,[10] the ^{1}H and ^{13}C NMR spectra of **3** (Table 1) showed an additional carbon signal ($\delta_{C\text{-}1''}$ 116.5) corresponding to a nitrile group that was further identified

(19) Fradcarbazole B (**2**): yellow, amorphous powder, $[\alpha]_D^{21}$ +21 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 237 (3.31), 283 (3.64), 326 (3.17), 364 (3.10) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 297 (+9.8), 277 (+0.9), 248 (+19.7), 212 (−15.0) nm; IR (KBr) ν_{max}: 2929, 1676, 1588, 1456, 1345, 1315, 746 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS m/z 526.1926 $[M + H]^+$ (calcd for $C_{29}H_{28}N_5O_3S$, 526.1913).

(20) Fradcarbazole C (**3**): yellow, amorphous powder, $[\alpha]_D^{21}$ +28 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 228 (3.28), 283 (3.62), 326 (3.14), 363 (3.06) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 295 (+8.4), 261 (−1.4), 249 (+5.4), 243 (+2.0), 231 (+5.2), 209 (−17.0) nm; IR (KBr) ν_{max}: 2925, 2213, 1680, 1587, 1457, 1345, 1316, 1120, 745 cm^{-1}; HRESIMS m/z 492.2044 $[M + H]^+$ (calcd for $C_{29}H_{26}N_5O_3$, 492.2036).

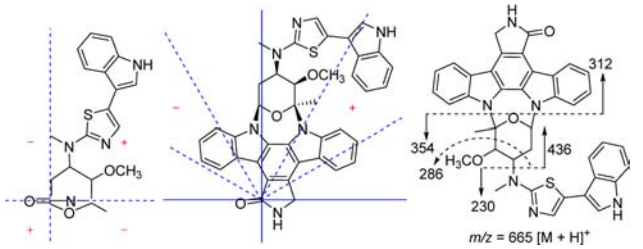
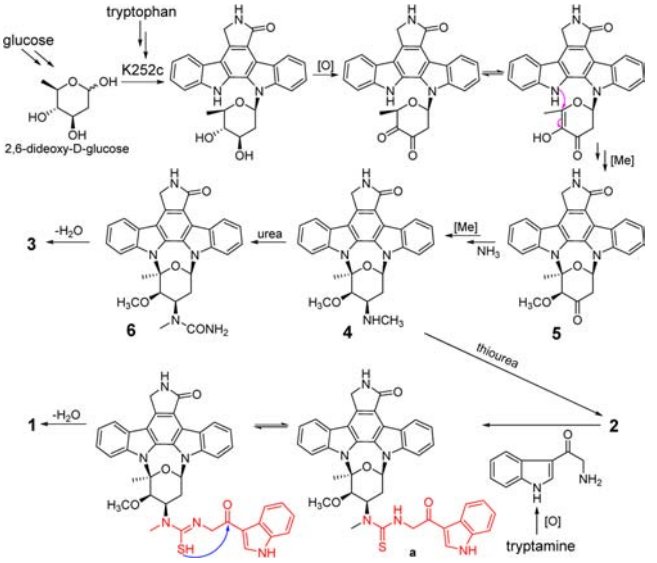


Figure 3. Application of an amide sector rule to **1** and Q-TOF-MS^2 sequence ions (m/z) for protonated molecular $[M + H]^+$ ion of **1**.

by the IR peak at 2213 cm^{-1}. The HMBC correlation from 3′-NCH_3 to C-1″ (Figure S2) supported this structure. The same relative configuration of **3** to staurosporine was deduced from the key NOE correlations of H-6′ to H-3′ and H-4′ and H-1′ to H-3′ (Figure S2). The similar CD Cotton effects of **3** to those of staurosporine (Figure 2) indicated the same absolute configuration. This deduction was further confirmed by the chemical transformation of staurosporine into **3** by the nucleophilic substitution of staurosporine with cyanogen bromide.[21] Thus, fradcarbazole C (**3**) was elucidated as 3′-*N*-cyanostauroporine.

Based on these trace amounts of ICZs, a plausible biosynthetic pathway for fradcarbazoles A−C (**1**−**3**) was postulated (Scheme 1). The biosynthetic intermediate **5** was derived from tryptophan and glucose.[22,23] Staurosporine (**4**) came from the amination followed by methylation of **5**, which may have underwent an amide exchange reaction with urea and thiourea to yield **2** and **6**, respectively. Compound **3** possibly resulted from the dehydration of **6**. Compound **2** may undergo an amide exchange reaction with β-oxotryptamine to yield an unisolated intermediate **a** whose thiole tautomer further underwent intramolecular aldol condensation followed by dehydration to yield **1**.

Scheme 1. Plausible Biosynthetic Pathway of **1**−**3**



Compounds **1**−**3** were assayed for their cytotoxic effects on HL-60, K562, A-549, and BEL-7402 cell lines and inhibitory effects on the kinase PKC-α by the MTT[24] and SRB method[25] and the Kinase-Glo luminescent kinase assay,[26] respectively. The results showed that **1**−**3** are strongly cytotoxic on HL-60/K562/A-549/BEL-7402 cell lines with IC_{50} values of 1.30/4.58/1.41/3.26, 1.60/1.47/0.001/1.74, and 0.13/0.43/0.02/0.68 μM, respectively, while the IC_{50} values of positive control adriamycin were tested as 0.65/0.64/0.08/0.37 μM, respectively. Compounds **1**−**3** and staurosporine also showed significant inhibition of PKC-α with IC_{50} values of 4.27, 0.85, 1.03, and 0.16 μM, respectively.

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Supporting Information Available. Experimental details, the NMR spectra of **1**−**3**, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(21) K_2CO_3 (7.2 mg, 0.052 mmol) was added to a solution of staurosporine (20 mg, 0.043 mmol) in dry THF (1 mL), and the mixture was stirred for 15 min at rt. Subsequently, cyanogen bromide (5.5 mg, 0.052 mmol) was added and the reaction was allowed to proceed for 3 h at rt. After evaporation of THF, the residue was added H_2O (7 mL) and extracted with EtOAc (3 × 3 mL). The organic layer was washed with brine (5 mL), dried with anhydrous Na_2SO_4, and concentrated to yield the EtOAc extract. Upon purification by HPLC over an ODS column, compound **3** (8.0 mg, 38% yield) was yielded as a pale yellow powder, which was identified by the same ESIMS, retention time in HPLC profile, and specific rotation as those of the natural one.

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