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Streptocarbazoles A and B, Two Novel Indolocarbazoles from the Marine-Derived Actinomycete Strain *Streptomyces* sp. FMA

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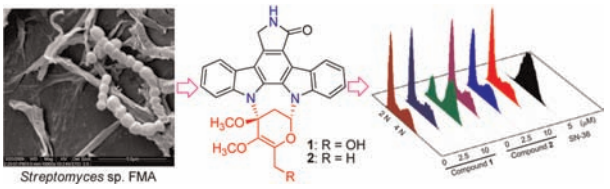
ABSTRACT



Streptocarbazoles A (1) and B (2), two novel indolocarbazoles featuring unprecedented cyclic *N*-glycosidic linkages between 1,3-carbon atoms of the glycosyl moiety and two indole nitrogen atoms of the indolocarbazole core, were isolated from the marine-derived actinomycetes strain *Streptomyces* sp. FMA. Their structures were established by spectroscopic methods, CD spectra, and ECD quantum mechanical calculations. Compound 1 was cytotoxic on HL-60 and A-549 cell lines and could arrest the cell cycle of Hela cells at the G_2/M phase.

During the last 35 years, more than 130 indolocarbazoles (ICZs) have been isolated from different organisms including bacteria, fungi, and invertebrates.[1] ICZs, kinds of alkaloids featuring a K252c[2] skeleton and represented by staurosporine,[3] have succeeded in attracting great attention from chemists, biologists, physicians, and pharmaceutical companies for their unusual structures and important biological activities.[4] Cytotoxicity,[5] protein kinase C (PKC),[6] and topoisomerase (Topo)[7] inhibitions are by far the most widely searched and reported biological activities for ICZs. Several ICZs, such as UCN-01,[8] lestaurtinib,[9] and NSC655649,[10] are currently being tested in the cancer clinical trials. As part of our ongoing research on new antitumor compounds from marine-derived actinomycetes,[11] strain FMA, identified as *Streptomyces*

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sp., was isolated from a mangrove soil collected in Sanya, Hainan Province of China. The EtOAc extracts of the fermentation broth of strain FMA exhibited significant cytotoxic effect on the P388 cell line. A series of metabolites contained in the extracts showed UV absorptions similar to those of ICZs at 227, 282, 325, and 355 nm[3,12] in an HPLC−UV profile (Figure S14, Supporting Information). Chemical investigation resulted in the isolation of two novel ICZs, which have been named the streptocarbazoles A (**1**) and B (**2**),[13] as well as five known analogues, K252c (indolocarbazole core),[2] K252a,[14] 3′-*epi*-K252a,[14] RK 286c,[15] and 4-bis(3-indolyl)-1*H*-pyrrole-2,5-dione.[16] Streptocarbazoles A (**1**) and B (**2**) possess exceptional cyclic *N*-glycosidic linkages between the 1,3-carbons of the glycosyl moiety and two indole nitrogen atoms of K252c. This connection was first discovered in the family of ICZs, and all 66 reported cyclic indolocarbazole glycosides were *N*-glycosidic linkages between the 1,5-carbons of the glycosyl moiety and the indolocarbazole core. In addition, there were only seven examples of cyclic *O*-glycosides in the literature, and the two glycosidic bonds were formed between the 1,2-carbons of the glycosyl moiety and the aglycone.[11b,17]

Streptocarbazole A (**1**) was assigned a molecular formula of $C_{28}H_{23}N_3O_5$ on the basis of HRESIMS,[18] requiring 19 degrees of unsaturation. Its UV spectrum showed a characteristic peak of indolocarbazole chromophore at λ_{max} 227, 282, 325, and 355 nm.[3,12] The IR absorption bands at 3329, 1681, 1590, and 1454 cm^{-1} suggested the presence of hydroxy, aromatic nucleus, and carbonyl groups. The ^1H and ^{13}C NMR spectra (Table 1) displayed signals similar to those of staurosporine,[19] further indicating an indolocarbazole unit that was confirmed by $^1H-^1H$ COSY of H-1/H-2/H-3/H-4 and H-8/H-9/H-10/H-11 and by 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, and H-11 to C-7c (Figure 1). Moreover, careful comparison of its ^1H and ^{13}C NMR spectra (Table 1) with those of staurosporine[19] revealed that the glycosyl moiety is different, and two olefinic quaternary carbons (δ_C 134.2 and 145.2) are present in the sugar moiety of **1**. This could be explained by the oxidation followed by enolization of the sugar moiety. $^1H-^1H$ COSY from H-1′ to H-2′ and the key HMBC

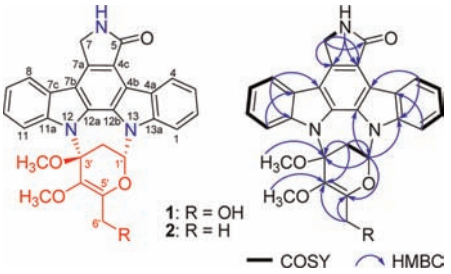

Figure 1. Structures and key $^1H-^1H$ COSY and HMBC correlations of **1** and **2**.

correlations of H-2′ to C-1′, C-3′ and C-4′, 3′-OCH_3 to C-3′, 4′-OCH_3 to C-4′, H-6′ to C-4′ and C-5′, and H-1′ to C-3′, C-5′ and C-12b suggested that the glycosyl moiety is 1,3-disubstituted 3,4-di-*O*-methyl-2-deoxy-4,5-dehydropyranoglucose and the glycosides are formed via the linkages of $C_{1'}-N_{13}$ and $C_{3'}-N_{12}$. *Cis*- and *trans*- could be two possible candidate relative configurations of **1**.

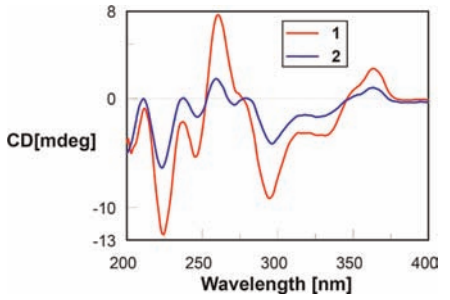

Figure 2. CD spectra of streptocarbazoles A (**1**) and B (**2**).

Model building of each via HyperChem 7.5 soft MM+ energy minimization calculations suggested that *trans*-**1** possessed significant intraring strain and higher molecular energy (Figure S1, Supporting Information). Furthermore, chemical shifts of ^{13}C NMR were computed at the B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d) level.[20] The magnetic shielding values were converted into chemical shifts after the corrections using slope and intercept of the linear-square functions,[20] and the relative errors of chemical shifts were computed by subtracting the calculated ^{13}C NMR from the experimental shifts. The relative errors between the measured ^{13}C shifts and the calculated ^{13}C shifts are less than 7.2 ppm in *cis*-**1**, while the maximum error at C-5′ reached 14.9 ppm in *trans*-**1** (Table S2, Supporting Information). Thus, the relative configuration of **1** was deduced as *cis*.

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(18) Streptocarbazole A (**1**): yellow, amorphous powder, $[\alpha]^{21}_D$ −54 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.32), 282 (3.65), 325 (3.15), 355 (3.09) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 364 (+1.5), 292 (−4.3), 259 (+4.1), 246 (−2.7), 237 (+0.6), 224 (−6.1), 212 (+1.5), 202 (−3.5) nm; IR (KBr) ν_{max} 3329, 2936, 1590, 1454, 1398, 1354, 1330, 1139, 1099, 1057, 1021, 746 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 482.1715 $[M + H]^+$ (calcd for $C_{28}H_{24}N_3O_5$, 482.1716).
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Table 1. ^{1}H and ^{13}C NMR Data for Streptocarbazoles A (**1**) and B (**2**) (600, 150 MHz, DMSO-d_6, TMS, δ ppm)

	1		**2**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	110.5, CH	7.82, d (8.3)	110.3, CH	7.82, d (8.2)
2	126.5, CH	7.55, t (8.3)	126.5, CH	7.55, t (8.2)
3	121.1, CH	7.35, t (7.8)	121.1, CH	7.36, t (7.8)
4	126.2, CH	9.37, d (7.8)	126.3, CH	9.37, d (8.2)
4a	123.4, C		123.4, C	
4b	116.3, C		116.3, C	
4c	120.1, C		120.1, C	
5	172.3, C		172.3, C	
6-NH		8.65, s		8.67, s
7	45.8, CH_2	4.98, s	45.8, CH_2	4.99, s
7a	134.2, C		133.3, C	
7b	116.9, C		116.8, C	
7c	123.5, C		123.5, C	
8	121.6, CH	8.03, d (7.8)	121.6, CH	8.03, d (7.8)
9	121.2, CH	7.35, t (7.8)	121.1, CH	7.36, t (7.8)
10	126.5, CH	7.53, t (8.2)	126.5, CH	7.52, t (8.2)
11	115.4, CH	8.23, d (8.7)	115.4, CH	8.24, d (8.2)
11a	141.1, C		141.1, C	
12a	133.9, C		133.9, C	
12b	125.5, C		125.5, C	
13a	139.7, C		139.6, C	
1′	77.5, CH	7.04, d (5.1)	77.6, CH	7.04, d (6.0)
2′	37.9, CH_2	3.41, dd (14.5, 6.0); 3.57, d (14.5)	37.8, CH_2	3.42, dd (15.6, 5.9); 3.55, d (15.6)
3′	87.8, C		87.8, C	
4′	134.2, C		134.0, C	
5′	145.3, C		142.9, C	
6′	56.3, CH_2	3.68, d (12.8); 3.82, d (12.8)	14.8, CH_3	1.59, s
3′-OCH_3	51.6, CH_3	3.56, s	51.6, CH_3	3.57, s
4′-OCH_3	64.0, CH_3	3.28, s	62.1, CH_3	3.14, s

An extension of the lactone sector rule[21] could be used to determine the absolute configuration of **1**. 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′*S*)-**1**. The functional group at C-3′ lying in the back upper left sector was responsible for the negative Cotton effect (π–π^* transition of conjugated lactam), which was good in accordance with the measured negative CD Cotton effect at λ_{max} 292 nm of **1** (Figure 2). Moreover, the quantum chemical ECD calculation method[22] was used to confirm the absolute configuration of **1**. The preliminary conformational distribution search was performed by HyperChem 7.5 software. The corresponding minimum geometries were further fully optimized by using DFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 03 program package. The stable conformers obtained were submitted to ECD calculation by the TDDFT [B3LYP/6-31G(d)] method. The overall predicted ECD spectrum of **1** was subsequently compared with the measured one. The measured

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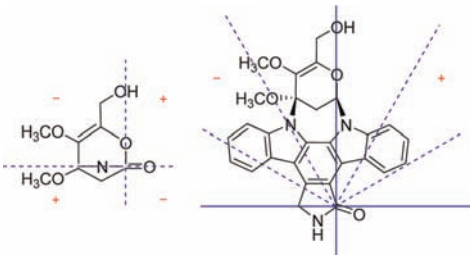

Figure 3. Application of an amide sector rule to **1**.

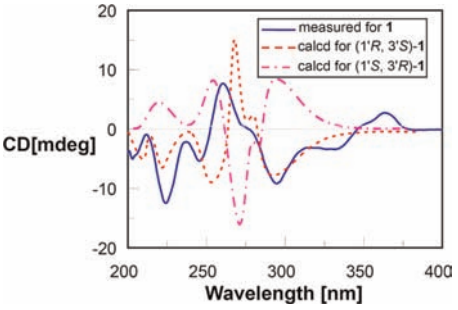

Figure 4. Measured and calculated ECD spectra for **1**.

CD curve of **1** showed Cotton effect at $\lambda_{max}(\Delta\varepsilon)$ 292 (−4.3), 259 (+4.1) and 224 (−6.1) nm, matching with the calculated ECD curve of (1′*R*, 3′*S*)-**1** and opposite to that of (1′*S*, 3′*R*)-**1** (Figure 4), and the B3LYP/6-31G(d)-optimized conformations were also used in optical rotation ($[\alpha]_D$) computations at the B3LYP/6-311G(d,p) level.[23] The calculated $[\alpha]_D$ value for (1′*R*, 3′*S*)-**1** was −56.6, close to the measured value of −54. Thus, the absolute configuration of **1** was unambiguously established to be (1′*R*,3′*S*).

The molecular formula of streptocarbazole B (**2**) was assigned to be $C_{28}H_{23}N_3O_4$ based on the HRESIMS,[24] which was only one oxygen less than that of **1**. Careful comparison of its ^{1}H and ^{13}C NMR spectra (Table 1) with those of **1** showed that a methyl signals at $\delta_{C/H}$ 14.8/1.59 in **2** replaced the corresponding hydroxymethyl signals at $\delta_{C/H}$ 56.2/3.68 and 3.82 in **1**, suggesting that the glycosyl moiety in **2** is 6-deoxygenated. The HMBC correlations from H_3-6′ to C-5′ and C-4′ also supported this change in **2** (Figure 1). The CD Cotton effects at $\lambda_{max}(\Delta\varepsilon)$ 292 nm ($\Delta\varepsilon$ −1.4), 259 nm ($\Delta\varepsilon$ +1.2) and 224 nm ($\Delta\varepsilon$ −2.4) (Figure 2) and the specific rotation ($[\alpha]_D$ −23) were almost

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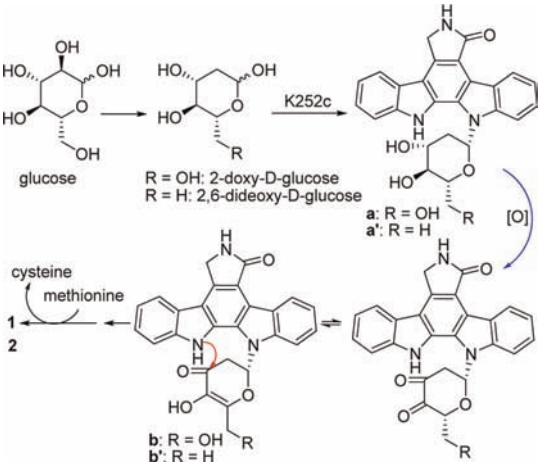
(24) Streptocarbazole B (**2**): yellow, amorphous powder, $[\alpha]^{21}_D$ −23 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.30), 282 (3.58), 325 (3.13), 355 (3.05) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 364 (+0.4), 292 (−1.4), 259 (+1.2), 246 (−0.8), 236 (+0.3), 224 (−2.4), 211 (+0.7), 200 (−2.2) nm; IR (KBr) ν_{max} 2924, 1632, 1454, 1387, 1354, 1330, 1295, 1144, 1062, 1020, 747 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 466.1758 $[M + H]^+$ (calcd for $C_{28}H_{24}N_3O_4$, 466.1767).

Scheme 1. Plausible Biosynthetic Pathway of **1** and **2**



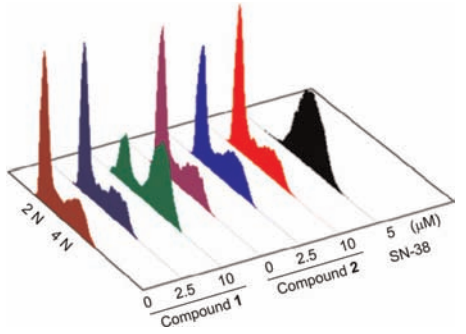


Figure 5. DNA histograms of Hela cells treated with different concentrations of streptocarbazoles A (**1**) and B (**2**).

the same to those of **1**, indicating the same absolute configuration. Thus, the structure of streptocarbazole B (**2**) was clearly elucidated as 6′-deoxystreptocarbazole A.

A plausible biogenetic pathway for streptocarbazoles A (**1**) and B (**2**) was postulated (Scheme 1). The indolocarbazole unit (K252c) was derived from tryptophan,[25] while the glycosyl moiety was probably developed from 2-deoxy-D-pyranoglucose. The glycosidation of 12-NH of K252c with 2-deoxypyranoglucose produced glycoside **a** that serves the key biosynthetic precursor. Glycoside **a** underwent an oxidation and an enolization to yield another glycoside **b** which further underwent aldol condensation followed by methylation to yield **1**. Streptocarbazole B (**2**) was probably formed from K252c and 2,6-dideoxy-D-pyranoglucose by the same biogenetic procedures.

Compounds **1** and **2** were tested for cytotoxic effects on the HL-60, A-549, and P388 cell lines using the SRB method[26] and on the Hela cell line using the MTT method.[27] The results showed that compound **1** is effective on HL-60, A-549, P388 and Hela cells, with IC_{50} values of 1.4,[13] 5.0,[13] 18.9, and 34.5 μM, respectively. And compound **2** was active against P388 and Hela cells with IC_{50} values of 12.8 and 22.5 μM, respectively, but no effects on HL-60 and A-549 cells were observed ($IC_{50} > 50$ μM). The inhibitory effects of **1** and **2** on the cell cycle[28] and the kinase[29] were also evaluated. The results showed that compound **1** arrested the cell cycle of Hela cells in the G_2/M phase at a concentration of 10 μM (Figure 5) and both new compounds did not show obvious inhibitory effects on the kinase GSK3-beta, Aurora-A, PKC-beta and PKC-epsilon (Table S1, Supporting Information).

Compounds **1** and **2** had been applied for the invention patents in China for the novel structures and the cytotoxicity against HL-60 and A-549 cell lines.[13] In this paper, the absolute configurations were revised and the bioactivities such as inhibition of cell cycle and the kinases and the cytotoxicity against Hela and P388 cell lines were complemented.

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Supporting Information Available. Experimental details, the NMR spectra of **1** and **2**, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.