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NOTES

A New Sesterterpene, Sch 599473, from a Marine Sponge, *Ircinia* sp.

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Marine organisms are an excellent source of structurally diverse molecules which are potentially useful for drug discovery.[1] Several marine natural products have been discovered as lead compounds in various therapeutic areas, and some of them are currently in clinical studies.[2,3] In our marine natural product research program, we have built a marine fraction library (MFL) for high throughput screening (HTS) for a variety of biological targets.[4] The active fractions are followed up using bioassay-guided fractionation to identify the active compounds. Isolation and identification of CCR7 receptor binding inhibitors from the marine sponge *Ircinia* sp. are described in this paper.

Chemokines and their receptors play important roles in many biological cascades, including allergic, inflammatory and metastatic processes. As such, small molecule agonists and antagonists of chemokine receptors have many potential therapeutic applications. The chemokine receptor, CCR7, is activated by CCL19 and CCL29 and regulates the mobilization of T cells and dentritic cells into the T cell areas of secondary lymphoid organs.[5] Therefore, CCR7 mediates an essential event for antigen-specific T cell activation and an important mechanism for mounting an immune response.[6] Thus, CCR7 could be a potential target for treatment of various metastases and/or inflammatory conditions.

In this study, a fraction from a sponge was identified which showed inhibitory activity in the CCR7 receptor binding assay. Followed by bioassay-guided purification of this active fraction, a new compound Sch 599473 (**1**) was discovered in addition to the principal active compound, sulfircin (**2**).

The sponge is an undescribed species of the genus Ircinia (Class Demospongiae, Order Dictyoceratida, Family Ircinidae).[7] The sponge was collected by dredging at a depth of 214 ft from the Gulf of Mexico (latitude 24°45.64′N, longitude 83°41.26′W). In life, the sponge was flabellate, fleshy, and easy to tear. It had a conulose surface. Its color, both alive and preserved in ethanol, is black externally and tan internally. It has knobbed filaments that are characteristic of the family, and the fibers in the ectosome incorporate a moderate amount of debris. A taxonomic reference specimen is deposited in the Harbor Branch Oceanographic Museum (catalog number 003:01003).

In the primary HTS assay, a sample in the MFL from a marine sponge *Ircinia* sp. inhibited [^{35}S]GTPγS exchange in Ba/F3-hCCR7 membrane at 20 μg/ml. Therefore, the active fraction was subjected to further purification. The preliminary CG161 fractions were prepared as previously described.[4,8] In this study, fraction 3 (50% acetonitrile (ACN) elution) was active in the CCR7 binding assay. Fraction 3 (35 mg) was further purified on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 20 mm×250 mm). The column was eluted with an aqueous ACN gradient system (15 ml/minute, 2% to 30% ACN over 40 minutes, and 30% to 70% ACN over next 40 minutes), to yield ~100 fractions (13 ml/fraction). Two pure compounds **2** (1.8 mg) and **1** (0.9 mg) were obtained with retention time ~54 minutes and ~57 minutes, respectively.

Detailed NMR analysis[9] idetified **2** as sulfircin, a compound whose structure had been determined previously by X-ray crystallography.[10]

The structure of **1** was determined based on extensive NMR and HRMS analyses and by comparison to **2**.[11] From the high-resolution negative ion ESI-MS, the molecular formula of **1** was established as $C_{25}H_{39}O_5S$ (found *m/z* 451.2529; calcd. 451.2523 for $[M]^-$). All spectral data including the HRMS, ^{1}H and ^{13}C NMR data strongly suggested that **1** was structurally related to **2**. The ^{13}C chemical shifts observed for C-17 (δ 124.7), C-18 (δ

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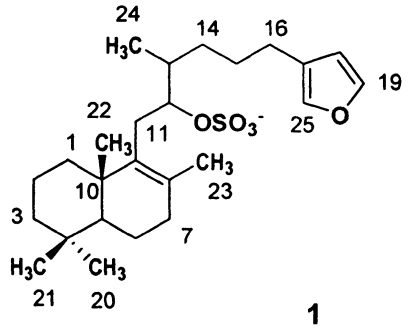
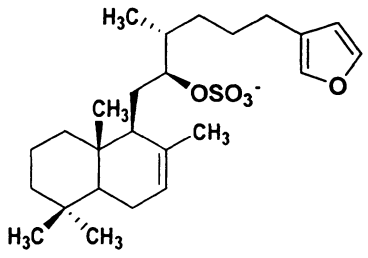
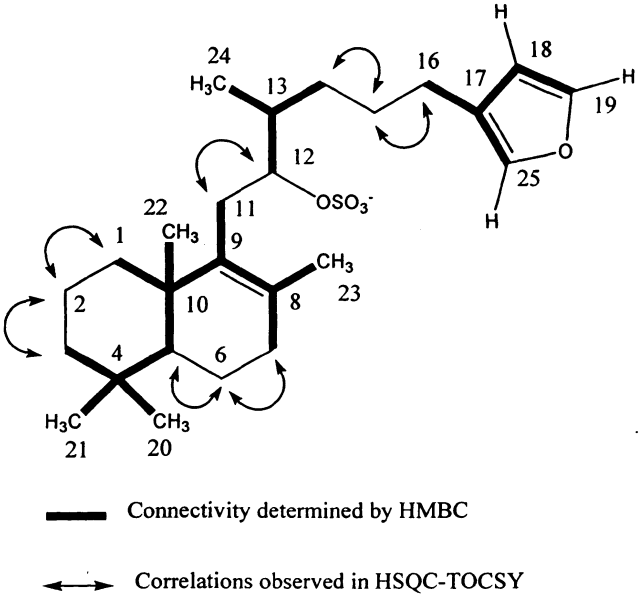


1



Sulfircin (2)

Fig. 1. Key HMBC and HSQC-TOCSY correlation of **1**.



111.1), C-19 (δ 142.9), and C-25 (δ 138.9) were typical for a 2-alkyl furan moiety. This was confirmed by HMBC correlations of H_2-16 (δ 2.35), H-18 (δ 6.35), H-19 (δ 7.53), and H-25 (δ 7.41) to the neighboring carbons as shown in Figure 1 and Table 1. The bicyclic ring skeleton was primarily determined by interpretation of the HMBC and HSQC-TOCSY spectra. For instance, the protons of the three methyl groups, H_3-20 (δ 0.85, s), H_3-21 (δ 0.80, s), and H_3-22 (δ 0.90, s), have significant long range correlations to C-5 (δ 50.8), suggesting the connectivity of C-20 (δ 33.2), C-21 (δ 21.7), and C-22 (δ 20.6) as shown by the bolded bonds in Figure 1. Both H_3-22 and H_3-23 (δ 1.51, s) had long-range correlations with C-9 (δ 137.3), suggesting the partial structure from C-22 through C-23 as shown by the bolded bonds in Figure 1. The connectivity of C-1 through C-3 and C-5 through C-7 was further determined from the HSQC-TOCSY spectrum from the following observations: correlations of H-1 (δ 1.14) to C-2 (δ 18.7); H-2 (δ 1.40, 1.50) to C-1 (δ 36.9) and C-3 (δ 41.4); H-5 (δ 1.07) to C-6 (δ 18.7); H-6 (δ 1.36, 1.58) to C-5 (δ 50.8) and C-7 (δ 33.4).

Observation of a correlation in the HSQC-TOCSY experiment between H_3-24 (δ 0.86) and C-13 (δ 34.8) established this connectivity. HMBC correlations observed between H_3-24 and a methane carbon (C-13), a methylene carbon (C-14, δ 31.0), and an oxygenated methine (C-12, δ 79.8) assign the latter two carbons as being adjacent to C-13. Correlations observed in the HSQC-TOCSY spectrum between H_2-11 (δ 2.06, 2.30) and C-12 and H-12 (δ 4.27) and C-11 (δ 28.5) firmly established the C-11-C-12 connectivity. Observation of a correlation between H_2-11 and both C-8 (δ 127.0) and C-9 in the HMBC spectrum defined the attachment of the C-11 methylene (δ 28.5) at C-9. Connectivity of C-14 through C-16 was determined by interpretation of the HSQC-TOCSY spectrum as shown in Figure 1. Therefore the sulfate group could only be located at the C-12 position and the planar structure of Sch 599473 (**1**) was unambiguously determined.

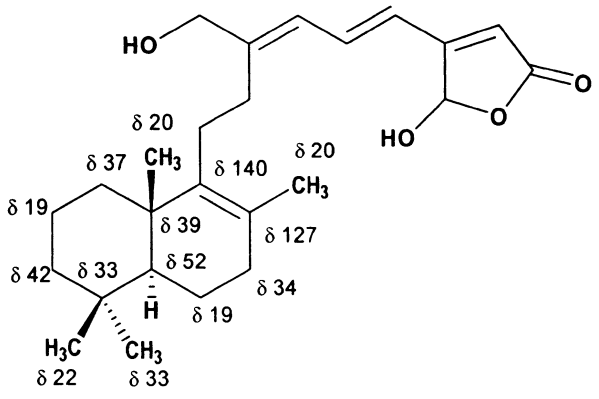
Trans configuration of the bicyclic structure in **1** was identified by its NMR data as well as in comparison with the NMR data to those of the model compounds, luffarins,[12] which have the identical bicyclic skeleton as **1** and their stereochemistry on the bicyclic ring have been established.[12~14] The coupling pattern of H-5 (dd, J=12.5, 2.0 Hz) in **1** indicated that H-5 was in the axial position. The same coupling pattern (H-5, dd, J=12.7, 2.0 Hz) was observed in the ^{1}H NMR of luffarin E (**3**). This type of coupling pattern would not be observed when the bicyclic

Table 1. NMR spectral data for compounds Sch 599473 (**1**) in DMSO-d_6.

C/H no.	^{1}H (δ)	^{13}C (δ)	Some HSQC-TOCSY (2 bond)	Important HMBC correlations
1	1.14, m	36.9 t	C-2	
	1.89, m			
2	1.40, m	18.7 t	C-1, C-3	
	1.50, m			
3	1.08, m	41.4 t	C-2	
	1.34 m			
4		33.1 s		
5	1.07, dd, J = 12.5, 2.0	50.8 d	C-6	
6	1.36, m	18.7 t	C-5, C-7	
	1.58, m			
7	1.90, m	33.4 t	C-6	
	1.97, m			
8		127.0 s		
9		137.3 s		
10		38.5 s		
11	2.06, brdd J = 14.6, 7.4	28.5 t	C-12	C-8, C-9
	2.30, brdd J = 14.6, 7.4			
12	4.27, ddd, J = 7.4, 7.4, 2.2	79.8 d	C-11	
13	1.82, m	34.8 d	C-14	
14	1.06, m; 1.40 m	31.0 t	C-13, C-15	
15	1.34, m	27.9 t	C-14, C-16	
	1.60, m			
16	2.35, t, J = 7.6	24.5 t	C-15	C-17, C-18, C-25
17		124.7 s		
18	6.35, s	111.1 d	C-19	C-17, C-19, C-25
19	7.53, s	142.9 d	C-18	C-17, C-25
20	0.85, s	33.2 q		C-3, C-4, C-5, C-21
21	0.80, s	21.7 q		C-3, C-4, C-5, C-20
22	0.90, s	20.6 q		C-1, C-5, C-9, C-10
23	1.51, s	20.5 q		C-7, C-8, C-9
24	0.86, d, J = 6.6	15.6 q	C-13	C-12, C-13, C-14
25	7.41, s	138.9 d		C-18, C-19

δ in ppm; J in Hz

Fig. 2. Structure and some ^{13}C NMR data (in $CDCl_3$) of luffarin E (**3**).



ring possesses *cis* configuration based on the 3D model analysis (H-5, estimated J=5 and 2 Hz from PCModel, v7.5). In addition, the ^{13}C chemical shifts of the bicyclic carbons in **1** matched very well with those of the model compound, luffarin E (**3**),[12] as shown in Figure 2. Therefore, the relative stereochemistry of the bicyclic ring was assigned to *trans* configuration.

The stereochemistry at C-12 and C-13 positions could not be defined by NMR methods. However, based upon the similarity of the NMR spectra of **1** and **2**, it is highly likely that they share the same stereochemistry.

Compound **2** exhibited fairly potent inhibitory activity in the CCR7 receptor binding assay. The IC_{50} values of **1** and **2** binding to the CCR7 receptor were 33 and 1.1 μM, respectively. The significant decrease of the activity of **1** in comparison to **2** indicated that the double bond shift from Δ7,8 to Δ8,9 significantly affects CCR7 binding.

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