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4849F, a New Metabolite Produced by the *Streptomyces* sp. 4849 as an Inhibitor of IL-4 Receptor

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Abstract A new compound named 4849F was isolated from the *Streptomyces* sp. 4849. The structure of 4849F was elucidated by spectroscopic analyses. The immobilized ligand-binding assay showed that 4849F can competitively inhibit the binding of IL-4 with IL-4 receptor in a dose dependent manner with an IC_{50} value of 6.7 μM.

Keywords 4849F, IL-4, IL-4R, inhibitor

IL-4 is a pleiotropic type I cytokine produced by activated T cells, mast cells, and basophils [1, 2]. It is considered important in the development of allergic inflammation and airway hyperresponsiveness (AHR). The overlap of its function results from the IL-4R-chain forming an important functional signaling component of both the IL-4 and IL-13 receptors [3]. Consequently, inhibition of the receptor-ligand interaction may offer an effective way to interfere the biological effect of IL-4. In the course of searching for IL-4 receptor inhibitors, a novel compound, 4849F (Fig. 1) was isolated from the *Streptomyces* sp. 4849 which had been deposited in CCGMC. In this paper, we described fermentation, isolation, structure elucidation and bioactivity of 4849F.

A slant culture of the *Streptomyces* sp. 4849. was maintained on an agar slant. After incubation at 28°C for 7 days, the slant was inoculated into 50 ml medium composed of yeast extract 0.5%, glucose 0.5%, tryptone 0.5%, beef extract 0.5%, corn steep liquten 0.4%, soybean extract 1.0%, starch 2.0%, $CaCO_3$ 0.4%. in 500-ml Erlenmeyer flasks. The fermentation was performed on a rotary shaker with 250 rpm at 28°C for 4 days. The filtrate (5 liters) obtained by filtration of fermentation broth was applied to an Amberlite XAD-5 column (4.5×35 cm), washed with distilled water and eluted with Me_2CO - H_2O (1 : 1). The eluted material was concentrated and then extracted with EtOAc. The EtOAc extract was concentrated and applied to a silica gel column (2.5×30 cm) and eluted with $CHCl_3$ - EtOAc (5 : 1). The first yellow band was further separated using the silica gel column (2×20 cm) eluted with cyclohexane - EtOAc (2 : 1). Pure 4849F was obtained in an amount of 4.0 mg.

The physico-chemical properties of 4849F are shown in Table 1. The UV spectrum of 4849F showed absorption maximum at 270 nm. The molecular formula of 4849F was established to be $C_{14}H_{12}O_2N_2$ by HR ESI-MS. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectral data of 4849F are shown in Table 2.

The ^{1}H-NMR spectrum showed methoxy protons (δ 4.18), and aromatic protons (δ 7.10, 7.74 and 8.00). The ^{13}C-NMR showed seven signals among which six are aromatic carbons and one is methoxy carbon. When considered together, molecular formula and the NMR signals indicated 4849F had a symmetrical structure which had two same phenyl group and methoxy group.

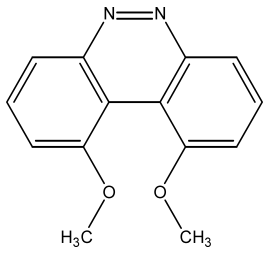

Fig. 1 Structure of 4849F.

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Table 1 Physico-chemical properties of 4849F

Appearance	Yellow needles
Melting point	233~235°C
Molecular formula	$C_{14}H_{12}O_2N_2$
HR ESI-MS (*m/z*)	
Found $(M+H)^+$	241.0971
Calcd	241.0977
UV λ_{max}^{MeOH} nm	270

Table 2 ^1H- and ^{13}C-NMR spectral data of 4849F recorded in $CDCl_3$

Position	^1H	^{13}C
1, 8	8.00 (d, *J*=8.0 Hz)	122.1
1a, 8a		143.0
2, 7	7.74 (t, *J*=8.0 Hz)	130.1
3, 6	7.10 (d, *J*=8.0 Hz)	106.9
4, 5		154.9
4a, 5a		136.9
4-OCH_3, 5-OCH_3	4.18 (s)	56.5

Connectivities from C-1 to C-2, and C-2 to C-3 were established by the ^1H-^1H COSY spectrum. The elucidated structure of 4849F was similar to the skeleton of phenanthrene which contained a structure of pyridazine. Given the simplicity of the NMR data presented, there were really only two possibilities for the site of attachment of the methoxy groups, given the proton coupling pattern observed in the COSY spectrum of the compound, either at C-4 or at C-1, since the three aromatic protons were contiguous. Furthermore, from the HMBC correlation data we knew, if the methoxy group was at the site of C-1 (δ 154.9), it should show the connection of H (δ 7.74) with C-1 (δ 154.9), C-4a (δ 136.9) and C-1a (δ 143.0). In fact it only showed the connection of H (δ 7.74) with C-1 (δ 154.9) and C-1a (δ 143.0). It was 4-bond coupling between H (δ 7.74) and C-1a (δ 143.0), but only 3-bond coupling between H (δ 7.74) and C-4a (δ 136.9). So the methoxy group must be at the site of C-4. The structure of 4849F was showed in Fig. 2.

The bioactivity of 4849F was evaluated in the immobilized ligand-binding assay method. The preparation of reagents and experimental procedures have been described in detail by Zhang [4]. Briefly, 100 μl of 2.0 μg/ml IL-4 was coated onto a 96-well immunoplate overnight at 4°C, then with 250 μl/well of 3.0% BSA in PBS (0.05 M phosphate buffer, pH 7.2, 0.15 M NaCl) for 8

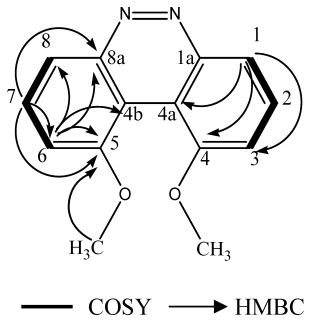

Fig. 2 ^1H-^1H COSY and HMBC correlations of 4849F.

hours at 4°C, followed by washing with PBST (0.1% Tween 20 in PBS). 50 μl samples were mixed with 50 μl sIL-4R. Then 100 μl of the mixtures were added to the plate and incubated overnight at 4°C. 100 μl/well of mouse monoclonal anti-human IL-4R antibody (diluted in PBSA) were added, incubated at 4°C for 1 hour. Then 100 μl/well of 1 : 1000 dilution of horseradish peroxidase (HRP)-labeled horse monoclonal anti-mouse IgG (H+L) in PBST was added and incubated as above. After the final wash, 100 μl/well TMB solution (3,3′,5,5-tetramethyl benzidine dihydrochloride) was added to each well and reaction took place at room temperature for 1 hour at room temperature. 100 μl of 2 N HCl were added to end the reaction. The absorbance at 450 nm was recorded as measurement of the reaction. 4849F inhibited the binding of IL-4 to IL-4R in a dose dependent manner with an IC_{50} value of 6.7 μM.

4849F has no activity for both of Gram-positive bacterias and Gram-negative bacterias. The MTT assays showed that the novel compound 4849F has cytotoxicities for human breast cancer MCF-7 and human ovarian cancer A2780 cell lines with IC_{50} 6.8 μM and 5.6 μM respectively [5]. Other bio-activities of 4849F are under investigation.

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