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# Sch 213766, A Novel Chemokine Receptor CCR-5 Inhibitor from *Chaetomium globosum*

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**Abstract** A novel fungal secondary metabolite, Sch 213766 was isolated from the fungal fermentation broth of *Chaetomium globosum* as the chemokine receptor CCR-5 inhibitor and shown to be the methyl ester of the previously described tetramic acid Sch 210972 on the basis of UV, MS and NMR spectral data analyses. Sch213766 exhibited an $IC_{50}$ value of 8.6 $\mu$M in the CCR-5 receptor *in vitro* binding assay.

**Keywords** anti-HIV, CCR-5 inhibitor, *Chaetomium globosum*, structure elucidation

Acquired immunodeficiency syndrome (AIDS) pandemic, which is caused by the type 1 human immunodeficiency virus (HIV-1) infection, has become one of the leading causes of death worldwide, and the number one cause in Africa. According to the recent report on AIDS epidemic update from UNAIDS (a joint United Nation program on HIV/AIDS) and WHO (World Health Organization), an estimated 34.1~47.1 million people were infected with HIV, 3.6~6.6 million became newly infected with HIV, and 2.9~3.5 million AIDS patients died at the end of 2006 [1]. The current standard medical treatment for HIV infected individuals called highly active antiretroviral therapy or HAART consists of three approved antiretroviral drugs, typically two reverse transcriptase inhibitors and one protease inhibitor to suppress HIV infection and reduce morbidity and mortality. Although HAART has proven effective to reduce viral load in patients with continuous dosing for over three years, this drug combination approach has not yet illustrated the capability of complete viral elimination from an infected individual. In addition, long-term toxicity and adverse drug–drug interactions of commercially available anti-HIV drugs are major concerns in medical treatments [2]. Furthermore, the emergence of viral resistance to protease and reverse transcriptase inhibitors has been reported due to the increasing use of antiretroviral agents [3]. Therefore, searching for effective anti HIV-1 infection agents with new mechanisms of action has become an urgent need. Recent studies indicated that binding to specific, cell surface co-receptors is an essential process before HIV-1 enters the targeted cells of the immune system. The chemokine receptor CCR-5 on macrophages, monocytes and T-cells, which belongs to the super family of seven-trans-membrane G-protein coupled receptors (GPCRs), has been identified as the surface co-receptor with the $CD_4$ molecule for viral entry [4]. The endogenous ligands of the CCR-5 receptor are the

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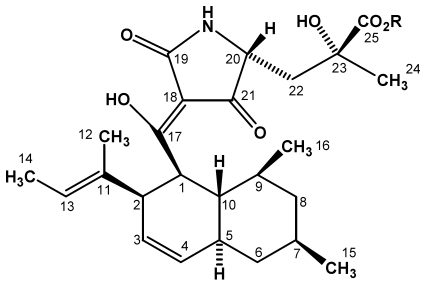

Sch 213766 (**1**) R = $CH_3$
Sch 210972 (**2**) R = H

$\beta$-chemokines MIP-1$\alpha$, MIP-1$\beta$ and RANTES [5]. Functional inhibition of the chemokine receptor CCR-5 is expected to block viral entry as an initial stage of HIV-1 infection. Therefore, discovery of potent and selective CCR-5 antagonists would potentially lead to a new anti-HIV drug [6].

To search for novel CCR-5 inhibitors as potential leads for development, a high throughput screening (HTS) campaign of extracts derived from various sources including actinomycets, fungal or plant species has been launched utilizing the CCR-5 membrane binding assays. Previously, the screening of these extracts had led to the discovery of two secondary metabolites as selective CCR-5 inhibitors produced by a fungal culture designated as *Chaetomium globosum* (Mer-0229) [7]. This report described the discovery of the methyl ester of Sch 210972 designated Sch 213766 (**1**), its production, isolation, structure elucidation, and biological activity.

The producing culture was collected and isolated from sterilized leaves of evergreen plants collected in Tucson, Arizona, USA. It was characterized as a fungus, *C. globosum*, based on morphological studies and deposited in the American Type Culture Collection with the accession number ATCC 74489.

The fermentation broth (4.0 liters) was adjusted with concentrated HCl to pH 2, and then extracted with 12 liters of EtOAc. After removal of solvent under reduced pressure, the crude extract (8.0 g) was partitioned between hexane : EtOAc : MeOH : $H_2O$ with 5% acetic acid, 8 : 2 : 5 : 5. The CCR-5 active concentrated complex from upper phase portion (800 mg) was chromatographed by normal phase HPLC (YMC semi-preparative PVA-Sil column 30×250 mm with guard 30×75 mm, S-5, 2.0% MeOH in *n*-butyl chloride, 24 ml/minute, UV=295 nm) to obtain crude **1**. The mixture was further purified through bioassay-guided fractionation by reverse phase HPLC (YMC semi-preparative ODS column, 20×250 mm with a guard column 20×50 mm, S-5, 70～100% acetonitrile in $H_2O$ with a linear gradient in 15 minutes, 12 ml/minute, UV= 220 nm) to afford pure **1** (20 mg) as gum-like material. **1** was soluble in acetone, EtOAc, acetonitrile, MeOH and $CH_2Cl_2$, but was insoluble in hexane and $H_2O$.

**Table 1** $^1$H- and $^{13}$C-NMR Data of Sch 213766 (**1**)[a]

| # | $^1$H | $^{13}$C |
|---|---|---|
| 1 | 3.95 dd (8.0, 7.0)[b] | 45.2[c] d[d] |
| 2 | 3.00 dt (8.0, 1.0) | 48.7 d |
| 3 | 5.66 br s | 126.2 d |
| 4 | 5.66 br s | 134.3 d |
| 5 | 1.82 m | 38.4 d |
| 6 | 0.95, 1.90 m | 42.4 t |
| 7 | 1.63 m | 33.2 d |
| 8 | 0.83, 1.66 m | 46.8 t |
| 9 | 1.39 m | 35.1 d |
| 10 | 1.40 m | 39.9 d |
| 11 | — | 138.2 s |
| 12 | 1.59 br s | 16.5 q |
| 13 | 5.19 dq (6.5, 1.0) | 122.0 d |
| 14 | 1.50 br d (6.5) | 13.8 q |
| 15 | 0.91 d (6.5) | 22.8 q |
| 16 | 0.84 d (6.5) | 20.2 q |
| 17 | — | 194.7 s |
| 18 | — | 100.9 s |
| 19 | — | 176.6 s |
| 20 | 3.80 dd (10.0, 2.5) | 60.7 d |
| 21 | — | 192.9 s |
| 22 | 1.75 dd (14.0, 10.0); 2.22 dd (14.0, 2.5) | 43.0 t |
| 23 | — | 75.3 s |
| 24 | 1.46 s | 28.0 q |
| 25 | — | 176.9 s |
| $OCH_3$ | 3.77 s | 52.9 q |

[a] Measured at 400 MHz in acetone-$d_6$, chemical shifts in ppm from TMS. [b] Coupling constants in Hz. [c] Recorded at 100 MHz in acetone-$d_6$, chemical shifts in ppm from TMS. [d] Multiplicity was determined by APT data.

Mass spectral data generated from LC/MS experiments using electrospray ionization (ESI) mode showed $M+H^+$ at *m*/*z* 460 indicating the molecular weight of 459 for **1**, which was also confirmed with FAB-MS data. The molecular formula was deduced by elemental analysis; calcd for $C_{26}H_{37}NO_6$: C 67.97, H 8.06, N 3.05%, found C 67.11, H 7.88, N 3.67%. UV absorption at 220 and 295 nm revealed the presence of a conjugation system indicating tetramic acid in **1**. Absorption bands in the IR spectrum at 3407, 1736 and 1655 $cm^{-1}$ suggested the presence of hydroxyl, carboxyl, and carbonyl groups, respectively. The $^{13}$C-NMR spectrum contained 26 carbon resonances

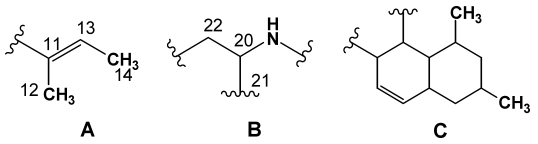


**Fig. 1** Partial structure assignments of Sch 213766 (**1**) based on COSY and HMQC data.

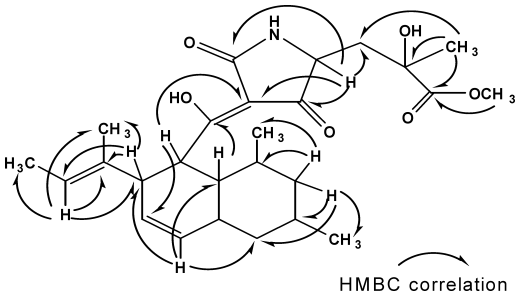


**Fig. 2** Important $^{1}$H-$^{13}$C long range coupling HMBC data of **1**.

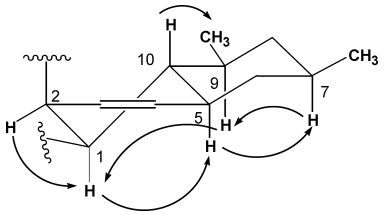


**Fig. 3** Some important NOESY data of **1**.

representing two carbonyl, two amide/acid carbonyl, two olefinic quaternary, three vinyl methine, one oxygenated quaternary, one nitrogenated methine, six methine, three methylene and six methyl carbons (Table 1). The $^{1}$H-NMR spectrum was consistent with $^{13}$C-NMR data showing three vinyl proton signals at $\delta$ 5.66 (H-3), 5.66 (H-4) and 5.19 (H-13), one nitrogenated methine signal at $\delta$ 3.80 (H-20), two allylic methine resonances at $\delta$ 3.95 (H-1) and 3.00 (H-2), two sets of a geminal methylene doublet of doublet (AB spin system) at $\delta$ 2.22 (H-22) and 1.75 (H-22), one allylic methyl singlet at $\delta$ 1.59 ($CH_3$-12), one allylic methyl doublet at $\delta$ 1.50 ($CH_3$-14), two methyl singlets at $\delta$ 1.46 ($CH_3$-24) and 3.77 ($CO_2CH_3$) and two methyl doublets at $\delta$ 0.91 ($CH_3$-15) and 0.84 ($CH_3$-16). Further 2D-NMR analysis with $^{1}$H-$^{1}$H correlation data permitted the establishment of three partial structures, A, B and C based on COSY and HMQC experiments as shown in Fig. 1. The assignments of these three fragments were accomplished by analysis of long range $^{1}$H-$^{13}$C correlation data generated from HMBC experiment. As shown in Fig. 2, the correlations of H-13 to C-2, as well as H-2 to C-11, C-12 and C-13 indicated attachment of fragment A to fragment C at position-2. The correlation of H-20 to C-18, C-19 and C-21 suggested the formation of a 2,4-pyrrolidine-dione ring from fragment B as a core unit of tetramic acid. The carbon chain extension on the methylene group ($CH_2$-22) attached to a hydroxyl-carboxylic bi-substituted two carbon moiety was assigned on the basis of correlations of H-24 to C-22, C-23 and C-25. The methyl ester functionality was assembled by analysis of the correlation of the $OCH_3$ ($\delta$ 3.77, s) to C-25. The final connectivity of the decalin moiety and tetramic acid through the enol carbon C-17 was established by HMBC correlations of H-1 to C-18, as well as H-10 to C-17. Thus, the structural assignments of all protons and carbons for **1** were completed, establishing **1** as the methyl ester of Sch 210972 (**2**), which was isolated previously from the same microorganism [7].

The relative configuration of **1** was determined by NOESY data (Fig. 3) and optical rotation data in comparison with **2**. The observation of four 1,3-diaxial couplings between H-1 and H-5, H-5 and H-7, H-7 and H-9, and H-9 and H-1 revealed the $\alpha$-orientation of these protons. The coupling between H-1 and H-2 further showed the same $\alpha$-configuration (equatorial) for the H-2 proton. Moreover, observation of NOE between H-10 and $CH_3$-9 showed that the H-10 proton had the $\beta$-orientation, thus forming a *trans* configuration at the decalin ring junction. The configurations of two chiral centers at C-20 and C-23 of **1** were established to be the same as **2**, which was determined by X-ray crystallographic data analysis [7], by direct comparison of their very similar optical rotation data with $[\alpha]_D^{25}$ value of +33.3° (*c* 0.1, $Me_2CO$) for **1** *vs.* +40.0° for **2**.

**1** displayed significant inhibitory activity with an $IC_{50}$ value of 8.6 $\mu$M in the CCR-5 membrane-binding assay. In the specificity study, **1** was also tested in the CCR-2 binding assay[†], and there was no inhibitory activity observed at 100 $\mu$M concentration. Interestingly, as the

[†] A high throughput screen utilizing a CCR-5 membrane-binding assay identified inhibitors of RANTES binding. This assay utilized membranes prepared from NIH 3T3 cells expressing the human CCR-5 chemokine receptor, which has the ability to bind to RANTES, a natural ligand for the receptor. In a 96-well plate format, 14 $\mu$g (total protein) of membrane preparation and 0.05 nM of $^{125}$I-RANTES were incubated in the presence (or absence) of compound, fraction or extract for one hour. Compounds or extracts were serially diluted over a wide range of 0.001 to 1 $\mu$g/ml and tested in triplicates. Reaction cocktails were harvested through glass fiber filters, and washed thoroughly. The $IC_{50}$ data reported as the concentration required inhibiting 50% of total $^{125}$I-RANTES binding.

methyl ester of **2**, the potency of **1** decreased over 100 fold in comparison to **2** ($IC_{50}$ ~0.08 $\mu$M) as an inhibitor of CCR-5. The data strongly indicated that the free carboxylic acid group plays an important role in the receptor binding.

The novel secondary metabolite **1** belongs to the tetramic acid (2,4-pyrrolidine-dione) class of antibiotics [8]. Numerous natural occurring tetramic acids have been reported in the scientific literature including coniosetin [9], vancoresmycin [10], CJ-17,572 [11], CJ-21,058 [12], ascosalipyrrolidinone A [13], reutericyclin [14], crypotocin [15], xanthobaccin A [16], LL-49F233 [17], PF1052 [18], lydicamycin [19], MBP049 [20], equistin [21], and phomasetin [22]. Tetramic acid type antibiotics possess antimicrobial activity against various microorganisms including many resistant microbial pathogens [8]. To the best of our knowledge, **1** and **2** are the only natural tetramic acid metabolites possessing CCR-5 antagonistic activity to be reported in literature.

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