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NOTE

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Fungal Phenalenones Inhibit HIV-1 Integrase

Kazuro Shiomi, Ryosuke Matsui, Miki Isozaki, Harumi Chiba, Takahiro Sugai, Yuichi Yamaguchi, Rokuro Masuma, Hiroshi Tomoda, Tomoko Chiba, Hua Yan, Yoshihiro Kitamura, Wataru Sugiura, Satoshi Ōmura, Haruo Tanaka

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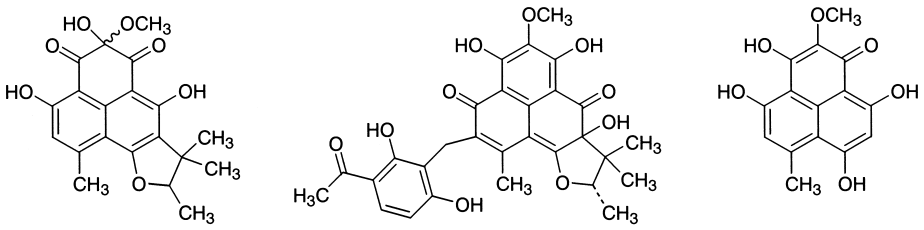
Abstract A phenalenone compound, atrovenetinone methyl acetal, was isolated from a culture broth of *Penicillium* sp. FKI-1463 as an HIV-1 integrase inhibitor, and it showed anti-HIV activity *in vitro*. HIV-1 integrase inhibition and anti-HIV activity of two other natural phenalenones were also studied. Among the tested compounds, funalenone inhibited HIV-1 integrase with an IC_{50} value of 10 μM and showed the best selectivity (anti-HIV, IC_{50}=1.7 μM; cytotoxicity, IC_{50}=87 μM).

Keywords: enzyme inhibitor, HIV interase, AIDS, phenalenone

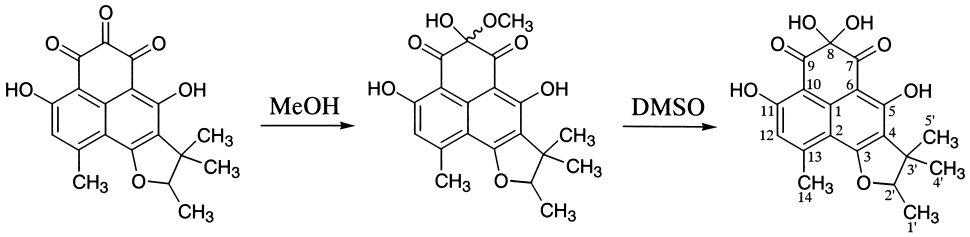
Combined therapeutic regimens with reverse transcriptase inhibitors and protease inhibitors lead to a suppression of human immunodeficiency virus-1 (HIV-1) replication, reduction of viral load, and decline in morbidity and mortality [1, 2]. However, the therapy sometimes fails due to the emergence of mutant viruses that are resistant to these drugs [3]. Thus, it is critical to discover more effective and less toxic anti-HIV agents with different molecular targets in the viral replication cycle. We have previously screened microbial metabolites for new anti-HIV antibiotics that inhibit entry of HIV-1 into the susceptive cells, and found isochromophilones and chloropeptins by a gp120-sCD4 binding assay [4, 5] and actinohivin by a syncytium formation assay [6]. There are three viral enzymes essential for HIV-1 replication, reverse transcriptase, protease, and integrase. Of these, only integrase has not been the target of a clinically used inhibitor. HIV DNA is inserted into the host genome by a specialized DNA recombination reaction in which the viral integrase is the key player [7, 8]. The integration reaction is composed of three steps, 3′-processing, strand transfer, and gap filling, and integrase catalyses the first and second steps. The third step is thought to be catalyzed by cellular enzymes. Many natural and synthetic integrase inhibitors have been reported [8～12] but only a few compounds show high selectivity. Therefore, we screened microbial metabolites for HIV-1 integrase inhibitors, and found that a culture broth of *Penicillium* sp. FKI-1463 has the inhibitory

H. Tanaka (Corresponding author)**, K. Shiomi, R. Matsui, M. Isozaki, H. Chiba, T. Sugai:** School of Pharmaceutical Sciences Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, E-mail: tanakah@pharm.kitasato-u.ac.jp
Y. Yamaguchi, R. Masuma, H. Tomoda, S. Ōmura: The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
R. Masuma, H. Tomoda, S. Ōmura: Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
T. Chiba, H. Yan, W. Sugiura: AIDS Research Center, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama-shi, Tokyo 208-0011, Japan
Y. Kitamura: Advanced Clinical Research Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan



Atrovenetinone methyl acetal (**1**) Erabulenol B (**4**) Funalenone (**5**)

Fig. 1 Natural phenalenones.



2 **1** **3**

Fig. 2 Conversion of atrovenetinone.

activity. The active compound was identified as a phenalenone compound, atrovenetinone methyl acetal (**1**, Fig. 1) [13]. This paper presents integrase-inhibiting and anti-HIV activities of **1** and other natural phenalenones.

A slant culture of the strain FKI-1463 grown on YpSs agar was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 2.0%, Polypepton (Nihon Pharmaceutical Co.) 0.5%, yeast extract 0.2% (Oriental Yeast Co.), KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and agar 0.1%, pH 6.0. It was cultured on a reciprocal shaker at 27°C for 3 days. One milliliter of the seed culture was transferred into each of twenty 500-ml Erlenmeyer flasks containing 100 ml of a production medium consisting of glycerol 3.0%, oatmeal (Nihon Shokuhin Seizo Co.) 2.0%, dry yeast (Gist-brocades) 1.0%, KH_2PO_4 1.0%, Na_2HPO_4 1.0%, $MgSO_4 \cdot 7H_2O$ 0.5%, pH not adjusted. The fermentation was carried out on a reciprocal shaker at 27°C for 7 days. The cultured broth (2.0 liters) was centrifuged and the mycelia were extracted with methanol, which was then removed from the extract by evaporation. The aqueous extract was partitioned with ethyl acetate at pH 3.0, and the organic layer was concentrated to dryness *in vacuo* to afford brown oil (644 mg). This was chromatographed over a silica gel column. Active fractions, eluted with $CHCl_3$-methanol (100 : 1) and $CHCl_3$-methanol (20 : 1), were concentrated to yield a crude material (284 mg). It was applied on a ODS silica gel column and eluted with aqueous CH_3CN. The 50% CH_3CN eluates were concentrated (95.5 mg) and chromatographed over Sephadex LH-20 to yield green oil (86.8 mg). It was further purified by reverse phase (Pegasil ODS, Senshu Scientific Co.) and normal phase (Pegasil Silica, Senshu Scientific Co.) HPLC to yield 50.5 mg of green oil.

The purified compound was implicated as **1** by comparison of the NMR data in $CDCl_3$ with the reported data by Nakanishi *et al.* [13]. Atrovenetinone (**2**) is easily converted into an acetal in alcohol (Fig. 2) [14], and the acetal is a mixture of diastereomers [13]. So, the NMR spectra of **1** are complicated. Since **2** exists as the hydrate (**3**) in DMSO [14], we observed the NMR spectra of the isolated compound in DMSO-d_6. The spectra were simplified, and each signal was assigned as follows: ^{1}H NMR (600 MHz) δ 13.67 (1H, s, 5-OH), 12.92 (1H, s, 11-OH), 6.86 (1H, s, 12-H), 4.70 (1H, q, J=6.5 Hz, 2′-H), 4.04 (1H, br s, 8-OH), 2.72 (3H, s, 14-H_3), 1.45 (3H, s, 5′-H_3), 1.22 (3H, s, 4′-H_3), 1.41 (3H, d, J=6.5 Hz, 1′-H_3); ^{13}C NMR (150 MHz) δ 197.7 (C-7), 196.2 (C-9), 165.1 (C-11), 164.8 (C-3), 164.5 (C-5), 147.9 (C-13), 136.7 (C-1), 118.1 (C-4), 117.6 (C-12), 109.0 (C-2), 104.9 (C-10), 101.9 (C-6), 91.1 (C-2′), 88.0 (C-8), 42.8 (C-3′), 25.2 (C-5′), 23.5 (C-14), 20.4 (C-4′), 14.3 (C-1′). The NMR data suggested that **1** was converted into **3** in DMSO solution (Fig. 2), and released methanol signals (δ_H 3.15 and δ_C 48.6) were also

Table 1 Biological activities of phenalenones

	IC_{50} (μM)			Selectivity (B/A)
	HIV-1 integrase inhibition	Anti-HIV activity (A)	Cytotoxicity (HPB-M(a)[a]) (B)	
Atrovenetinone methyl acetal (**1**)	19	6.7	13	1.9
Erabulenol B (**4**)	7.9	17	230	14
Funalenone (**5**)	10	1.7	87	51

[a] HPB-M(a) cells are human peripheral blood cells transformed by murine leukemia virus. Anti-HIV activity was measured using HPB-M(a) cells with LTR driven luciferase.

observed. Thus, the isolated compound was identified as **1**. It has been reported as a myosin light chain kinase inhibitor isolated from a culture broth of *Penicillium* sp. It may be derived from **2** during purification. Compound **2** is a phenalenone compound originally obtained by the oxidation of atrovenetin produced by *Penicillium* sp., and **2** was lately isolated from a culture broth of *Gremmeniella abietina* [14, 15].

We have previously isolated the other fungal phenalenones, erabulenol B (**4**) which inhibits cholesteryl ester transfer protein and funalenone (**5**) which inhibits collagenase [16, 17]. Funalenone was also reported to inhibit bacterial cell wall synthesis enzymes MraY and MurG [18]. We evaluated integrase inhibition and anti-HIV activity of **1** together with those phenalenones. HIV-1 integrase activity was measured by strand transfer assay according to Craigie *et al.* [7]. *In vitro* anti-HIV activities of the test compounds were measured by originally established reporter human T cell line with LTR driven luciferase. The cells were infected with wild type HIV-1, and the compounds were added at different concentrations ranging from 0.0016 to 125 μg/ml. Luciferase activities of the cells, which appeared to correlate with the level of HIV-1 replication, were measured at day 7, and anti-HIV IC_{50}s of the compounds were evaluated. The IC_{50} value of **1** against integrase was 19 μM, and it also showed anti-HIV activity at 6.7 μM (Table 1). However, its cytotoxicity was relatively high. Compounds **4** and **5** showed more potent inhibition against integrase than **1**, and also exhibited anti-HIV activity. The anti-HIV activity of **5** was the most potent (1.7 μM), and its cytotoxicity (87 μM) was lower than **1**. Though **5** was reported to inhibit collagenase and bacterial cell wall synthesis enzymes [17, 18], those inhibitions were less potent than the integrase inhibition and anti-HIV activity. Therefore, **5** may be a good candidate lead compound for anti-HIV agent. Inhibition of DNA polymerases by the other phenalenones have been reported, but they did not inhibit HIV reverse transcriptase [19]. A plant metabolite, hypericin [20], is the only *ortho*- and *peri*-fused aromatic compound reported to show integrase inhibition [21].

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