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Hexylitaconic acid: A new inhibitor of p53–HDM2 interaction isolated from a marine-derived fungus, *Arthrinium* sp.

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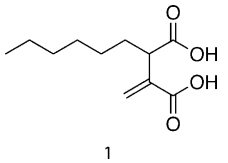
Abstract—A new inhibitor of p53–HDM2 interaction was isolated from a culture of marine-derived fungus, *Arthrinium* sp. The structure was identified to be (−)-hexylitaconic acid (**1**) by spectroscopic analysis. The inhibition of p53–HDM2 binding was tested by the ELISA method, and **1** inhibited the binding with an IC_{50} value of 50 μg/mL. Although a number of synthetic inhibitors of p53–HDM2 interaction have been reported so far, **1** is the second inhibitor isolated from natural resources.

Ubiquitination of proteins requires the sequential action of three enzymes, the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin–protein ligase (E3).[1,2] E3s are a large family of enzymes that recognize huge numbers of target proteins and destine them for degradation. As E3 definitively determines which target proteins are ubiquitinated, it can be inferred that a specific inhibitor against an E3 recognizing a key target protein could be a good lead for therapy of the disease connected with the turnover of the key target protein. Among many E3s, MDM2 (mouse double minute 2) or HDM2 (human double minute 2), an E3 for p53 protein,[3,4] is frequently used as a target for inhibitor development. HDM2 is normally expressed at a low level, while it is overexpressed in a variety of human cancers. p53 is a tumor suppressor that is mutated in more than 50% human cancers and this protein induces growth arrest and apoptosis upon activation by various stimuli such as DNA damage.[5] Therefore, targeting HDM2 is a promising approach to reactivate p53, inducing apoptosis in human cancer cells. In the course of our search for new classes of inhibitors against the ubiquitin-proteasome proteolytic pathway from natural resources, we already succeeded in isolating agosterol derivatives[6] and secomycalolides A[7] as proteasome inhibitors and himeic acid A[8] as an E1 inhibitor. In addition, we found that girolline, an anticancer compound, is the first agent inhibiting the recruitment of polyubiquitinated p53 to the proteasome.[9] In this paper, we describe the isolation of a new inhibitor of p53–HDM2 interaction, (−)-hexylitaconic acid (**1**), from a culture broth of a marine-derived fungus, *Arthrinium* sp., and its inhibitory activity against p53–HDM2 interaction (Chart 1).

The fungus, *Arthrinium* sp.,[10] was separated from a marine sponge, collected in Toyama Bay in the Japan Sea. The fermentation culture[11] (6.0 L) was separated into the mycelium and culture medium by filtration. The culture medium, which showed inhibitory activity against p53–HDM2 binding, was passed through a HP-20 column. The resin was washed with H_2O and then MeOH. The EtOAc soluble part (2.8 g) of the MeOH eluent was purified by ODS chromatography with aqueous MeOH. The fraction (107 mg) eluted with 60% MeOH was purified by ODS HPLC (YMC-Pack ODS, 5 μm, ϕ



1

Chart 1.

Keywords: p53; HDM2; Ubiquitin ligase; Marine-derived fungus; *Arthrinium* sp.
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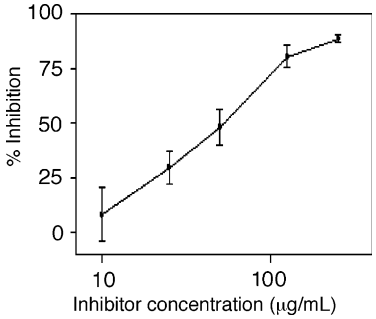

Figure 1. Inhibition of p53–HDM2 interaction by **1**. Data are expressed as means ± SE ($n = 3$).

20 × 250 mm, YMC Co., Ltd.; 80% MeOH–H_2O; flow rate, 4.0 mL/min; t_R 25 min) to afford an inhibitor (**1**, 75 mg)[12] of p53–HDM2 interaction. The compound **1** was identified to be (−)-hexylitaconic acid by comparing its spectroscopic data with the values previously reported.[13]

The inhibition of p53–HDM2 interaction was tested by ELISA according to the standard procedure using purified recombinant p53 and HDM2 proteins,[14] and the following primary anti-MDM2 antibody (Santa Cruz, SMP14). As shown in Figure 1, **1** inhibited p53–HDM2 interaction in a dose-dependent manner, and the IC_{50} value was determined to be 50 μg/mL. To analyze the structure–activity relationship, three derivatives of **1**, a mono methyl ester **2**, a dihydro derivative **3**, and a dihydro derivative of mono methyl ester **4**, were prepared[15] (Chart 2) and their inhibitory activities were measured. In contrast to **1**, however, **2**–**4** as well as two commercially available dicarboxylic acids, itaconic acid and succinic acid, did not inhibit the interaction at all at the concentration of 50 μg/mL.

(+)-Hexylitaconic acid was first isolated as a plant growth regulator from *Aspergillus niger* cultivated in field soil. This study was the first report on the isolation of hexylitaconic acid as a natural product.[13] Subsequently, the (−)-enantiomer was isolated as a metabolite produced by the marine endophytic fungus *Apiospora montagnei* isolated from the alga *Polysiphonia violacea*.[16] In addition, the isolation of hexylitaconic acid from the marine sponge-derived *Aspergillus niger*, of which the absolute configuration had not been mentioned, has also been reported so far.[17] However, no biological activity of hexylitaconic acid was reported in the two latter papers.

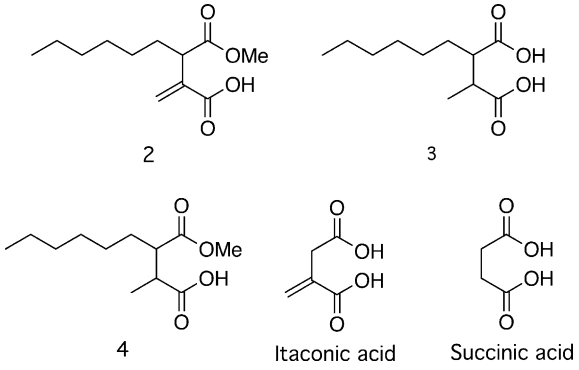

Chart 2.

The crystal structure of the 109-residue amino-terminal domain of MDM2 bound to a 15-residue transactivation domain peptide of p53 revealed that MDM2 has a deep hydrophobic cleft, to which the p53 peptide binds.[18] Thus, MDM2 inhibitors that can bind to the cleft and inhibit the binding between MDM2 and p53 could be good lead compounds for treatment of cancer. So far, various peptides have been designed for antagonists against MDM2, and non-natural amino acids were used as components in the peptides to enhance their binding to the MDM2 cleft.[19] In addition, several types of non-peptidic compounds have been reported.[20–23] Among them, nutlins[22] bind to the p53-binding pocket of HDM2 and block p53–HDM2 interaction in vitro and in vivo. Although a number of synthetic inhibitors of p53-MDM2 interaction have been reported so far, (−)-hexylitaconic acid (**1**) is the second inhibitor isolated from natural resources. The first compound, chlorofusin,[24,25] was isolated from a culture of *Fusarium* sp.

Acknowledgments

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10. The fungus was identified on the basis of the morphological evaluation by NCIBM Japan Co., Ltd. (Shizuoka, Japan). A voucher specimen is deposited at Kanazawa University with the code MF571.
11. The fungus was grown in a fermentation broth composed of 1:1 seawater/deionized water with 2.0% malt extract and 0.5% peptone without shaking at 25 °C for 3 months.

12. Compound **1**; $[\alpha]_D^{21}$ −8 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 0.89 (3H, t, J = 6.6 Hz), 1.30 (2H, m), 1.31 (2H, m), 1.32 (4H, m), 1.67 (1H, m), 1.83 (1H, m), 3.44 (1H, t, J = 7.6 Hz), 5.74 (1H, s), 6.32 (1H, s); ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.6 (CH_2), 28.6 (CH_2), 30.1 (CH_2), 32.3 (CH_2), 32.8 (CH_2), 48.0 (CH), 126.8 (C), 141.0 (C), 169.5 (C), 177.1 (C); FABMS *m/z* 215 $[M+H]^+$, 237 $[M+Na]^+$, 259 $[M-H+2Na]^+$, 281 $[M-2H+3Na]^+$.
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