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THREE BIOACTIVE COMPOUNDS AGAINST COLONY FORMATION OF CHINESE HAMSTER V79 CELLS FROM AN INDONESIAN ASCIDIAN *Didemnum* sp.

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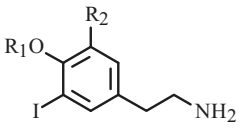
Marine invertebrates, such as sponges, ascidians, and soft corals, have been a rich source of bioactive chemical components with unique structural features [1, 2]. Ascidians (tunicates) have provided a number of interesting compounds with marked bioactivities and peculiar structures, and, among them, ecteinascidin 743 has been utilized for the treatment of soft-tissue sarcomas [3]. The characteristic feature of structures isolated from ascidians is the high contents of the nitrogenous compounds [4], which are divided into two major groups, peptides (and amino acids) and alkaloids as ecteinascidins.

In the course of our studies on the chemical components of ascidians collected in North Sulawesi, we have reported the isolation of two new tryptamine derivatives, leptoclinidamide and (–)-leptoclinidamine B, from the Indonesian ascidian *Leptoclinides dubius* [5] and found that the EtOH extract of *Didemnum* sp. showed remarkable activity on the colony formation of Chinese hamster V79 cells (100% inhibition at 50 μg/mL). This bioassay reflects the direct action of compounds on the cells. Bioassay-guided separation from the EtOH extract led to the isolation of three bioactive components, 4-(2-aminoethyl)-2-iodophenol (**1**) [6], 3,5-diiodo-4-methoxyphenethylamine (**2**) [7–9], and plakinidine D (**3**) [7, 9]. We describe herein the isolation, structure identification, and bioactivity of compounds **1**–**3**.

The EtOH extracts of 45 ascidians collected in the coral reefs at North Sulawesi in 2009 were tested for their effects on the colony formation of Chinese hamster V79 cells [10]. This bioassay reflects the direct action of samples on the cells. Seventeen extracts exhibited 100% inhibition on the colony formation of V79 cells at 50 μg/mL.

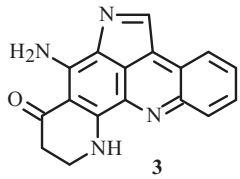
The EtOH extract of *Didemnum* sp. (Code No. 09M42), collected at the East side of Bunaken Island, inhibited the colony formation of V79 cells completely (100%) at 50 μg/mL and was separated by an ODS column into six fractions (Frs. 1–6). The bioactive Fr. 2 was purified by preparative HPLC and afforded 4.5 mg of compound **1**. Fraction 3 showed bioactivity and was subjected to preparative HPLC to yield compounds **2** (27.5 mg) and **3** (0.7 mg).

Compound **1,** a white powder, showed the molecular ion peak at *m/z* 263 in the EI mass spectrum. The ^{1}H NMR spectrum of **1** in CD_3OD revealed two methylene signals [δ 2.82 (2H, t, J = 7.7 Hz), 3.11 (2H, t, J = 7.7 Hz)] and three aromatic proton signals [δ 6.81 (1H, d, J = 8.1 Hz), 7.10 (1H, dd, J = 8.1, 1.8 Hz), 7.62 (1H, d, J = 1.8 Hz)] of a 1,2,4-trisubstituted benzene ring. These spectroscopic data suggested that compound **1** was an iodinated tyramine derivative. A literature search in SciFinder provided the structure of 4-(2-aminoethyl)-2-iodophenol as a candidate. Comparison of the spectroscopic data of **1** with the reported values [6] identified the structure of **1**.



1, 2

1: $R_1 = R_2 = H$
2: $R_1 = CH_3$, $R_2 = I$



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Compound **2,** a white powder, gave the molecular ion peak at *m/z* 403 in the EI mass spectrum. Two methylene proton signals were observed at δ 2.85 (2H, t, J = 7.5 Hz) and 3.13 (2H, t, J = 7.5 Hz) in the ^{1}H NMR spectrum of **2**, which were very similar to those of **1**. Remarkable differences in the ^{1}H NMR spectra between **1** and **2** were detected in the aromatic and methoxy proton signals. The ^{1}H NMR spectrum of **2** showed two aromatic protons at the same position [δ 7.75 (2H, s)] and a methoxy methyl proton signal at δ 3.80 (3H, s). From these spectroscopic data, the structure of **2** was identified as 3,5-diiodo-4-methoxyphenethylamine [7–9].

The molecular ion peak was observed at *m/z* 288 in the EI mass spectrum of **3** as an orange oil. The ^{1}H NMR spectrum of **3** in CD_3OD revealed two methylene signals [δ 2.94 (2H, t, J = 7.4 Hz) and 4.05 (2H, t, J = 7.4 Hz)] and five aromatic proton signals [δ 7.84 (1H, t, J = 7.6 Hz), 7.88 (1H, t, J = 7.6 Hz), 8.41 (1H, d, J = 7.6 Hz), 8.46 (1H, d, J = 7.6 Hz), and 8.57 (1H, s)], which suggested the presence of fused heteroaromatic rings in the molecule of **3**. A literature search in SciFinder based on the MS and NMR data indicated the structure of **3** as plakinidine D [7, 9].

The effects of compounds **1**–**3** were tested against the colony formation of V79 cells [10] and the growth of four microorganisms (*Mucor hiemalis*, *Saccharomyces cerevisiae*, *Candida albicans*, and *Escherichia coli*) [5]. Compounds **1**–**3** inhibited the colony formation of V79 cells with IC_{50} values of 13.2, 1.1, and 2.2 μM, respectively. On the other hand, compounds **1**–**3** did not show apparent activity against four microorganisms. Compound **3** was previously reported to show *in vitro* cytotoxicity against the human colon cancer cell line HCT-116 at 5 μg/mL [9].

This is the first report on the inhibitory activity of compounds **1**–**3** on the colony formation of V79 cells. Moreover, compound **1** was isolated for the first time from Indonesian ascidians.

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