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ORIGINAL ARTICLE

Sagitol C, a new cytotoxic pyridoacridine alkaloid from the sponge *Oceanapia* sp.

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Abstract A new pyridoacridine alkaloid named sagitol C (**2**) together with two known compounds; kuanoniamine C (**1**) and sagitol (**3**) were isolated from the EtOAc fraction of the Indonesian sponge *Oceanapia* sp. Their chemical structures were established on the basis of physical and spectroscopic methods 1D and 2D NMR, in addition to mass spectrometry and comparison with literature data. Sagitol C was found to exhibit cytotoxic activity when tested against different cancer cell lines.

1. Introduction

Oceanapia comprises more than 50 nominal species recorded for the Indo-west Pacific region alone, relatively abundant in both soft and hard substrates, and widely distributed.[1] Pyridoacridine alkaloids have been isolated from sponges, ascidians, anemones, and prosobranch.[2] They are characterised by 11*H*-pyrido[4,3,2-*mn*]acridine moiety and have so far only been reported from marine organisms. The colours exhibited by pyridoacridine alkaloids may vary depending on their pH. This physicochemical property is correlated with the presence of at least two basic nitrogens in the aromatic ring systems.[3] They are known to exhibit a wide range of biological activities including; anti-bacterial, anti-fungal, anti-viral, anti-parasitic, insecticidal, anti-tumour, topoisomerase inhibition, and anti-fouling.[4] *Oceanapia* species have yielded different classes of natural products including; alkaloids,[1–5] sphingolipid-like compounds,[6] sphingolipids;[7,8] polyhydroxy sterols,[9] acetylenes,[10] and dithiocyanates.[11] In the course of our investigation on bioactive compounds from the marine sponges, we have isolated a new pyridoacridine alkaloid named sagitol C (**2**), in addition to two known pyridoacridine alkaloids; kuanoniamine C (**1**) and sagitol (**3**) (Fig. 1). We have also investigated the effect of sagitol C (**2**) on the growth of three tumour cell

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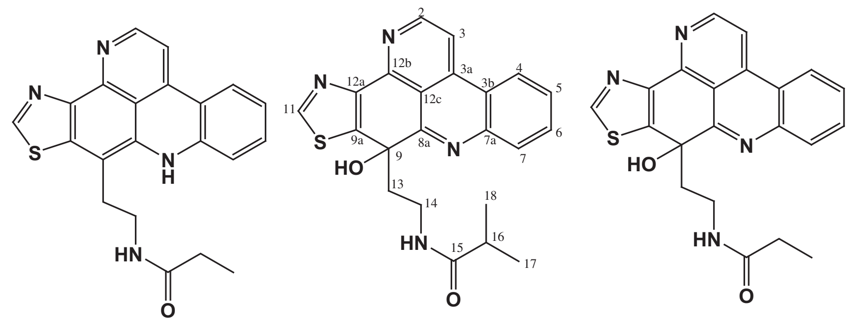

1 2 3

Figure 1 Chemical structures of the isolated compounds.

lines: mouse lymphoma (L5178Y), rat brain (PC12), and human cervix (Hela).

2. Experimental

2.1. General procedures

Mass spectra (ESIMS) were recorded on a Finnigan MAT TSQ-7000 triple stage quadrupole mass spectrometer. HRESIMS were measured on a Finnigan MAT 95 mass spectrometer. UV spectra were recorded in absolute MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on a Bruker DRX400 NMR spectrometer using standard Bruker software and DMSO-d_6 as solvent, with TMS as the internal reference. NMR spectra were referenced to the solvent signals (DMSO-d_6: 2.49 ppm for ^{1}H and 39.9 ppm for ^{13}C). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm), RP-18 (0.04–0.063 mm Merck), and Sephadex LH-20 (0.25–0.1 mm, Merck). TLC was performed on precoated TLC plates with silica gel 60 F_{254} (layer thickness 0.2 mm, Merck). The chromatograms were developed using the following solvent system: $CHCl_3$:MeOH (90:10, S_1). The compounds were detected by UV absorption at λ_{max} 255 and 366 nm followed by spraying with Dragendorff's reagent.

2.2. Animal material

The sponge was collected by scuba diving at a depth of 7–9 m from Ambon, Indonesia in 2004. Freshly collected sponges were frozen immediately after collection and then freeze-dried. The dull-red sponge has a fistular growth form, consisting of an irregular turnip-shaped main body up to 8 cm high and 4 cm in diameter, from which issue several long hollow fistules 7–8 cm long and up to 1 cm in diameter. The surface is optically smooth and slightly rough when dry. The consistency of the freeze-dried specimen is very fragile, crumbly, and dusty. The skeleton of the periphery is a halichondroid, tangential, multilayered crust of intercrossing single spicules. This is carried by thin subectosomal spicule tracts of 2–3 spicules in cross section, following a meandering course and anastomosing irregularly. The skeleton of the interior of the sponge is scanty, consisting of a loose irregular reticulation of mostly single spicules bound at the nodes with a little spongin. Spicules are exclusively curved, somewhat flexuous oxeas with variously pointed or blunt apexes that are uniform in size 260–330 × 3–6 μm.[3] It was kindly identified by Prof. Rob W.M. van Soest (Zoological Museum, University of Amsterdam). A voucher specimen is kept under registration no. ZMA POR 11007 in the Zoölogisch Museum, Amsterdam.

2.3. Extraction and isolation

The freeze-dried sponge (110 g) was extracted several times with a ratio of 1:1 CH_3Cl:MeOH (1 L × 4). The total extract was evaporated to dryness (398 mg) and subjected to vacuum liquid chromatography (VLC) over silica gel using *n*-hexane, EtOAc, and MeOH as solvents. The EtOAc fraction (164 mg) was chromatographed over Sephadex LH-20 column (150 g × 50 × 2 cm) using MeOH as an eluent. 50 mL fractions were collected and monitored by TLC to obtain three subfractions OE-1–OE-3. Subfraction OE-1 (39 mg) was subjected to RP-18 column (0.04–0.063 mm; 40 g × 25 × 1 cm) using MeOH:H_2O gradients to give **1** (7 mg, yellow amorphous powder). Subfraction OE-2 (57 mg) was chromatographed over silica gel column (100 g × 50 × 3 cm) using $CHCl_3$:MeOH gradient to obtain impure compounds **2** and **3**. Separately, the impure **2** and **3** were purified on RP-18 column (0.04–0.063 mm; 40 g × 25 × 1 cm) using MeOH:H_2O gradients to give **2** (3 mg, orange residue) and **3** (5 mg, orange residue).

Kuanoniamine C (**1**): It was isolated as yellow amorphous powder (7 mg); R_f = 0.78 (S1). UV (MeOH) λ_{max}: 267 and 358 nm; ESIMS *m/z*: 375 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.4 (1H, brs, NH), 9.41 (1H, s, H-11), 8.49 (1H, brs, H-2), 8.28 (1H, dd, J = 8.2, 1.8 Hz, H-4), 7.88 (1H, brs, H-3), 7.71 (1H, dd, J = 8.2, 1.8 Hz, H-6), 7.71 (1H, brd, J = 8.2 Hz, H-7), 7.25 (1H, dd, J = 8.2, 1.8 Hz, H-5), 3.20 (2H, m, H-14), 3.15 (2H, m, H-13), 2.07 (2H, q, J = 7.6 Hz, H-16), 0.97 (3H, t, J = 7.6 Hz, H-17). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 174.6 (C-15), 153.2 (C-11), 143.5 (C-12b), 143.0 (C-2), 140.7 (C-12a), 140.4 (C-7a), 140.2 (C-9a), 139.4 (C-3a), 135.0 (C-6), 133.5 (C-8a), 125.4 (C-4), 122.8 (C-5), 118.3 (C-12c), 117.6 (C-7), 114.3 (C-3b), 108.2 (C-3), 107.7 (C-9), 36.3 (C-13), 30.9 (C-14), 28.4 (C-16), 9.7 (C-17).

Sagitol C (**2**): It was isolated as orange residue (3 mg); R_f = 0.71 (S1). UV (MeOH) λ_{max}: 254 and 366 nm. NMR data: see Table 1; HRESIMS *m/z*: 405.1464 (calcd for $C_{22}H_{21}N_4O_2S$, $[M+H]^+$, 405.1463).