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Microxine, a New cdc2 Kinase Inhibitor from the Australian Marine Sponge *Microxina* Species

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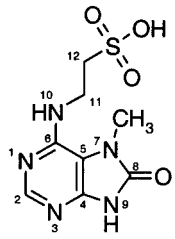
A new purine derivative microxine (**1**) was isolated from the Australian marine sponge *Microxina* sp. The compound was isolated via reversed-phase chromatography and its structure determined spectroscopically. Microxine was found to weakly inhibit cdc2 kinase activity with an IC_{50} of 13 μM.

A number of compounds containing substituted purines have been isolated from sponges including purine nucleosides,[1−3] guanine derivatives,[4] adenine derivatives such as 3-methyladenine,[5] and the 9-methyladeninium-containing *Agelas* metabolites ageline A and B,[6] agelasine,[7] and agelasine A−F.[8,9] Recently the compound erinacean (**2**) has been reported from the Antarctic sponge *Isodictya erinacea*.[10] In our continuing search for biologically active marine metabolites we have isolated a cdc2 kinase inhibitor, microxine from the Australian marine sponge *Microxina* sp. Upon the basis of spectral data, the structure of this compound has been established as **1**.



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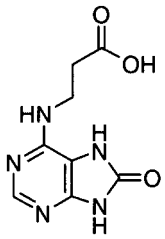

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As part of a program aimed at the discovery of novel antiinflammatory agents, an ethanol extract of the lead organism, *Microxina* sp., was observed to inhibit the binding of ^{125}I-Bolton Hunter labeled C5a to its receptor. C5a is an 11 kD fragment of the fifth component of complement with potent anaphylatoxic and chemotaxic activities. Two compounds were isolated from the ethanol extract of this organism. The C5a-active compound was a complex high molecular weight compound (MW ~8200 amu) of undetermined structure, which was the source of the activity seen in the bioassay. This compound did not meet the criteria for continued work in this particular assay because of size and complexity, and thus the final structure was not defined. A second compound, trivially named microxine, was isolated as a byproduct of the separation and proved to be inactive in the C5a bioassay. Upon the basis of its structural similarity to other cdc2-active purine compounds such as 6-dimethylaminopurine, it was tested in the cdc2 kinase bioassay. Microxine gave activity in this assay with an IC_{50} of 13 μM.

Compound **1** was isolated from the ethanol extract of *Microxina* sp. via solvent partitioning followed by preparative reversed-phase HPLC. The ^{13}C NMR, DEPT, and HRFABMS spectra of **1** indicated a molecular formula of $C_8H_{11}N_5O_4S$ requiring six unsaturation equivalents. The NMR spectra of **1** in D_2O exhibited an A_2X_2 spin system [δ_H 2.95 (2H, t, J = 5.6 Hz), δ_C 49.0 (t); δ_H 3.70 (2H, t, J = 5.6 Hz), δ_C 37.4 (t)] consistent with a taurine functionality;[11] a downfield methyl singlet [δ_H 3.30 (3H, s), δ_C 28.6 (q)] consistent with an *N*-methyl functionality; a heteroaromatic methine [δ_H 7.96 (1H, s), δ_C 147.0 (d)]; and four downfield quaternary carbon resonances [δ_c 107.2 (s), 143.9 (s), 145.1 (s), and 153.9 (s)]. These data together with the UV for **1** suggested the presence of a substituted purine skeleton[2,12] and were most consistent with an adenine derivative.[5−10] Analysis of the HMBC data for **1** suggested that both the taurine substituent and the aromatic proton were on the A ring, as H-2 and the H-11 methylene protons exhibited long-range coupling to the carbon observed at δ 145.1 (C-6). These data preclude either substituent being in position C-8, as $^4J_{C6-H8}$ and $^4J_{C8-H6}$ have been shown to be very small (<1 Hz) for purine compounds.[12,13] The *N*-methyl group was placed in position 7 on the basis of long-range HMBC correlations observed to δ 107.2 (C-5) and 153.9 (C-8). This left two possible isomeric structures for **1**, a 7-methyl-8-oxo derivative with the taurine functionality at either C-2 or C-6. Placement of the taurine at C-6 was determined by analysis of the aromatic $^1J_{C-H}$ via a fully coupled HMQC experiment ($^1J_{C-H}$ = 204.3 Hz). It has been shown that the $^1J_{C-H}$ in a number of purine compounds are relatively independent of substitution and are diagnostic of substituent position.[12,13] For purine and a number of purine compounds substituted in the C-6 position, the reported mean value of $^1J_{C2-H2}$ is 203.9 ± 3.8 Hz (C-2 bears two nitrogen substituents), whereas for purine compounds substituted in the C-2 position, the reported mean $^1J_{C6-H6}$ is 185.1 ± 3.4 Hz[12] (C-6 bears only one nitrogen substituent). Additional data that support the assigned structure are the ^{1}H and ^{13}C NMR spectral data reported for erinacean (**2**), a related compound isolated from the sponge *Isodictya erinaea*.[10]

Cyclin-dependent kinases (cdk) play an essential role in the regulation of the cell division cycle. The p34^{cdc2}/cyclin

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B kinase is a highly conserved mitotic factor implicated in cell cycle transitions in all eucaryotic cells. A series of 32 substituted purine derivatives have recently been tested in the cdc2 kinase assay.[14] The IC_{50} values for these compounds ranged from 0.2 μM for 6-(benzylamino)-2-(*R*)-[[1-(hydroxymethyl)propyl]amino]-9-isopropylpurine (roscovitine) to >1000 μM. Microxine (**1**) proved to be active in this assay, with an IC_{50} of 13 μM. Microxine was assayed for its ability to inhibit in vitro growth of the P388 murine leukemia and A549 human adenocarcinoma tumor cell lines.[15] No inhibition of growth was detected in either assay at concentrations as high as 18 μM. Microxine was also tested for its ability to inhibit the protein phosphatase cdc25a, but no inhibition was observed at concentrations up to 10 μM.

Experimental Section

General Experimental Procedures. Spectral data were measured on the following instruments: FTIR Midac M-1200 with Galactic GRAMS/386 software; UV Perkin-Elmer Lambda 3B; NMR Bruker AMX-500 with the X-32 computer; MS Kratos MS-80RFA, FAB-NOBA (Chemical Instrumentation Center, Yale University). ^{1}H NMR chemical shifts are reported as δ values in ppm relative to HOD (4.63 ppm). ^{13}C NMR chemical shifts are reported as δ values in ppm relative to MeOH (48.9 ppm). ^{13}C multiplicities were measured using the DEPT sequence. One-bond ^{1}H−^{13}C connectivities were determined via the 2D proton-detected HMQC experiment. Multiple-bond ^{1}H−^{13}C connectivities were determined via the 2D proton-detected HMBC experiment optimized for a long-range ^{1}H−^{13}C coupling constant of 10 Hz.

Bioassay Protocol. Cdc2 kinase activity[14] and binding of [^{125}I]Bolton-Hunter labeled C5a to PMN membranes[16] were measured as previously described.

Animal Material. The sample was collected in Cape Jaffa, South Australia (latitude 36° 57′ S; longitude 139° 36′ E), on a rocky outcropping at a depth of 7 m using scuba.This sponge has been assigned as a new but still undescribed and unnamed species of the genus *Microxina* (class Demospongiae, order Haplosclerida, family Niphatidae). The voucher specimen is deposited in the Queensland Museum (accession number QM G300621).

The sponge is thickly encrusting, approximately 30−40 cm in diameter, yellow externally and internally, with parasitic *Parazoanthus* growing on the surface and embedded in the mesohyl. The surface is even, occasionally bumpy with large rounded conules and prominently microporous with microscopic ridges and bumps (“microconules”). Large areas of the surface are covered with filamentous algae, and algal filaments are scattered throughout the choanosome, occasionally clumped around larger fibers.

This species is assigned to the family Niphatidae because of its ectosomal structure (dense multispicular three-dimensional structure, in this case consisting of erect oxeas, 185−234 × 4−8 μm), significantly more compact than the underlying choanosomal skeleton (consisting of differentiated ascending and transverse connecting fibers/spicule tracts and interstitial spicules). Within this family, the present species fits closest with *Microxina* Topsent, although deviating from all other known species of the genus in having a mostly smooth (not markedly conulose) surface and in having sigmas (2 size classes, 13−28 × 0.8−1.5 μm and 58−74 × 1.5−2.5 μm) and rhaphide microscleres (112−135 × <0.5 μm) in addition to the usual microxeas (2 size classes, 28−39 × 1 μm and 64−83 × 1−2 μm). Rhaphides are definitely native to this species, not artifacts or contaminants, and as such they are highly unusual within the Haplosclerida.

Extraction and Purification. The diced sponge (50 g) was extracted by blending with EtOH (3 × 200 mL). This extract was dried under vacuum to obtain a yellow solid (2.1 g), which was partitioned between *n*-butanol and water. Aqueous partition (500 mg) was subjected to preparative reversed-phase HPLC (Rainin Dynamax Macro C_{18}, H_2O/CH_3CN gradient containing 0.1% TFA, 0 to 60% CH_3CN over 20 min) to yield **1** (3.0 mg, 0.006% of wet weight).

Microxine (1): white amorphous solid; UV (H_2O) λ_{max} (ϵ) 210 (12886), 274 (ϵ 8408) nm; IR (film on KBr) ν_{max} 3445 br, 1694, 1632, 1455, 1433, 1386, 1343, 1195, 1170, 1042, 736, 598 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) δ 2.95 (2H, t, J = 5.6 Hz, H-12), 3.30 (3H, s, 7-NMe), 3.70 (2H, t, J = 5.6 Hz, H-11), 7.96 (1H, s, H-2); ^{13}C NMR (D_2O, 125.7 MHz) δ 28.6 (q, 7-NMe), 37.4 (t, C-11), 49.0 (t, C-12), 107.2 (s, C-5), 143.9 (s, C-4), 145.1 (s, C-6), 147.0 (d, C-2), 153.9 (s, C-8); HRFABMS (3-nitrobenzyl alcohol) observed *m*/*z* 274.0666 [M + H]$^+$ (calcd for $C_8H_{12}N_5O_4S$ *m*/*z* 274.0610).

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