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# Turbinamide, a New Selective Cytotoxic Agent from the Mediterranean Tunicate *Sidnyum turbinatum*

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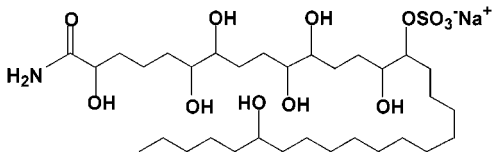
## ABSTRACT

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**A unique cytotoxic metabolite, turbinamide (1), has been isolated from the marine tunicate *Sidnyum turbinatum* through a bioassay-guided approach. Its structure has been elucidated by an extensive spectroscopic analysis. Turbinamide demonstrated a strong and selective cytotoxic effect against neuronal cells rather than immune system cells.**

Gliomas constitute the most malignant forms of brain tumors and are also considered among the most malignant forms of cancer. Although modern cancer chemotherapy makes it possible to cure completely and to increase the survival of patients affected by several kinds of tumors, the therapeutic treatment of gliomas is up to now only palliative.[1] It is therefore of considerable importance to find new molecules as antiproliferative agents that may form the basis of a potential new therapy for the treatment of this type of tumor.

As a part of our ongoing chemical and pharmacological studies of tunicates,[2] we have recently isolated some novel cytotoxic alkyl sulfates from *Sidnyum turbinatum* Savigny (Polyclinidae).[3] We now report the isolation, from the same ascidian, and structural elucidation of turbinamide (**1**), a new sulfated polyhydroxydotriacontanamide, which was shown to be a selective inhibitor of proliferation of cells from the nervous system in confront to cells from the immune system. In this paper we describe the chemical and pharmacological characterization of this unique compound. Exhaustive methanol extraction of specimens of *S. turbinatum*, collected in the Bay of Naples (Procida, Punta Pizzaco; 40 m depth) afforded a brown-colored crude extract that was partitioned between $H_2O$ and *n*-BuOH. The *n*-BuOH-soluble material, which showed a strong cytotoxicity in vitro, was subjected to a bioactivity-directed fractionation by MPLC on a $C_{18}$ stationary phase. The active fractions eluted with 1:1 $H_2O$/MeOH were further separated and purified by repeated $C_{18}$ reversed-phase HPLC (Luna C18 150 × 4.6 mm, 3 $\mu$m, 3:7 $H_2O$/MeOH) yielding turbinamide (**1**, 5 × $10^{-2}$ % dry weight).[4]

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(4) Turbinamide (**1**): $[\alpha]^{25}_D$ +7.1 (*c* 0.0031 in $CH_3OH$); FABMS (negative ion mode) *m*/*z* 686.2 $[M - Na]^-$.

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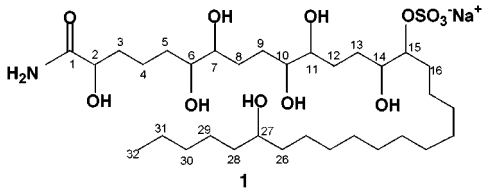
**Table 1.** NMR Data of Turbinamide (**1**)[a]

| pos | δC | δH, mult (*J* in Hz) | HMBC (H to C) | HMQC−HOHAHA[b] (H to C) | pos | δC | δH, mult (*J* in Hz) | HMBC (H to C) | HMQC−HOHAHA[b] (H to C) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 180.60 | | | | **13** | 28.48 | Ha: 1.77[c] | 15 | |
| | | | | | | | Hb: 1.61[c] | 14, 15 | |
| **2** | 72.35 | 4.03, m | 1, 3, 4 | 3, 4, 5, 6 | **14** | 75.73 | 3.61, bd (9.6) | 12, 13, 16 | 12, 13 |
| **3** | 35.57 | Ha: 1.68[c] | 2, 4, 5 | | **15** | 79.51 | 4.24, bdd (7.4, 13) | | |
| | | Hb: 1.80, m | 1, 2, 4, 5 | | | | | | |
| **4** | 22.42 | Ha: 1.70[c] | | | **16** | 33.08 | Ha: 1.65[c] | 15, 17 | |
| | | Hb: 1.51[c] | | | | | Hb: 1.46[c] | | |
| **5** | 33.42 | Ha: 1.45[c] | 3, 4 | 2, 3, 4, 6 | **17/25** | 30.84 | 1.32−1.36 | | |
| | | Hb: 1.63[c] | | | | | | | |
| **6** | 76.31 | 3.42[c] | 7, 8 | 7, 8, 9 | **26** | 38.40 | Ha: 1.39[c] | 27 | |
| | | | | | | | Hb: 1.45[c] | 27 | |
| **7** | 76.31 | 3.45[c] | 8, 9 | 6, 8, 9 | **27** | 72.28 | 3.52, m | | 26/28 |
| **8** | 30.11 | Ha: 1.42[c] | 7/10 | | **28** | 38.40 | Ha: 1.39[c] | 27, 29, 30 | |
| | | Hb: 1.92, bd (8.4) | 7/10 | | | | Hb: 1.46[c] | 27, 29, 30 | |
| **9** | 30.11 | Ha: 1.42[c] | 7/10 | | **29** | 26.60 | 1.32−1.36 | | |
| | | Hb: 1.92, bd (8.4) | 7/10 | | | | | | |
| **10** | 76.31 | 3.45[c] | 8, 9, 11 | 8, 9 | **30** | 33.08 | 1.32−1.36 | | |
| **11** | 75.55 | 3.48[c] | 10, 12, 13 | | **31** | 23.53 | 1.32−1.36 | | |
| **12** | 30.46 | Ha: 1.96, m | 10 | | **32** | 14.36 | 0.94, t (6.6) | 30, 31 | 28, 29, 30, 31 |
| | | Hb: 1.41[c] | | | | | | | |

[a] $^1$H (500.14 MHz) and $^{13}$C NMR (125.03 MHz) spectra were recorded in $CD_3OD$; chemical shifts were referred to the residual solvent signal ($\delta_H = 3.34$, $\delta_C = 49.0$). [b] Gronenborn, A. M.; Bax, A.; Wingfield, P.; Core, G. M.; *FEBS Lett.* 1989, *243*, 93. [c] Partially overlapped by other resonances.

The molecular formula of turbinamide (**1**) was determined as $C_{32}H_{64}NO_{12}SNa$ by HRFABMS (*m*/*z* 732.3950 [M + Na]$^+$, calcd value 732.3928), implying one degree of unsaturation. The presence of a sulfate group was inferred by the IR absorptions at $\nu_{max}$ 1248 and 1070 cm$^{-1}$ and supported by a characteristic deshielded oxygenated methine (δH 4.24, m; δC 79.51) found in the NMR spectra. The IR spectrum was also indicative of the presence of a carbonyl group ($\nu_{max}$ 1671 cm$^{-1}$), confirmed by a low field carbon signal (δ 180.60) present in the $^{13}$C NMR spectrum; these spectral data suggested the presence of an amide group, accounting for the only unsaturation indicated by the molecular formula of **1**.




**1**

The molecule contained seven more oxygen atoms in addition to those satisfying the sulfate and the carbonyl groups, as deduced from the molecular formula; they were arranged in seven secondary hydroxyl groups taking advantage of the favorable $^1$H and $^{13}$C NMR spectral features. In fact, seven oxymethine proton signals [δ 3.42, 3.45 (2H), 3.48, 3.52, 3.61, and 4.03] were observed in the $^1$H NMR spectrum, in addition to the above-reported signal at δ 4.24. They were associated, through an HMQC experiment, to the corresponding methine carbons resonating in the midfield region of the $^{13}$C NMR spectrum (see Table 1).

Once the functionalities of **1** had been established, an extensive analysis of 2D NMR data allowed their positioning in the molecule, whose linear structure was indicated by the $^{13}$C NMR spectrum, exhibiting in the high-field region only a series of methylene signals, apart from a methyl resonance at δ 14.36. Combined analysis of $^1$H−$^1$H COSY, HOHAHA, HMQC, HMBC, and HMQC−HOHAHA spectra was carried out with some difficulty deriving from some severely overlapping oxymethine and methylene signals in the $^1$H NMR spectrum. The connectivity patterns H2−H4 and H5−H16 were deduced from the $^1$H−$^1$H COSY spectrum. Partial overlapping of H4 and H5 methylene signals prevented the delineation of the whole spin sequence H2−H16, which was deduced from both HOHAHA and HMBC data. In fact, in the HOHAHA spectrum the spin system H2−H6 is unambiguously delineated; in addition, HMBC spectrum shows the key correlations between methylene protons $H3_a$ (δ 1.68) and $H3_b$ (δ 1.80) and the carbon signals at δ 22.42 and 33.42 (C-4 and C-5, respectively). Furthermore, a diagnostic HMBC cross-peak between H2 and the carbonyl at δ 180.60 made it possible to locate the amide at C-1 (see Table 1).

All of the above data gave sufficient evidence to identify the fragment C1−C16, the most functionalized portion of the molecule. A conclusive proof was provided by negative ion FAB-MS/MS measurements. As shown in Figure 1, the results obtained further supported the location of hydroxyl groups at C2 (*m*/*z* 641/611), C6 and C7 (*m*/*z* 569/539/509), C10 and C11 (*m*/*z* 481/451/421), and C14 (*m*/*z* 393/363). The remaining segment, C17−C32, was a hydrocarbon chain containing the still unallocated hydroxyl group; thus, the only structural information required to establish the structure of

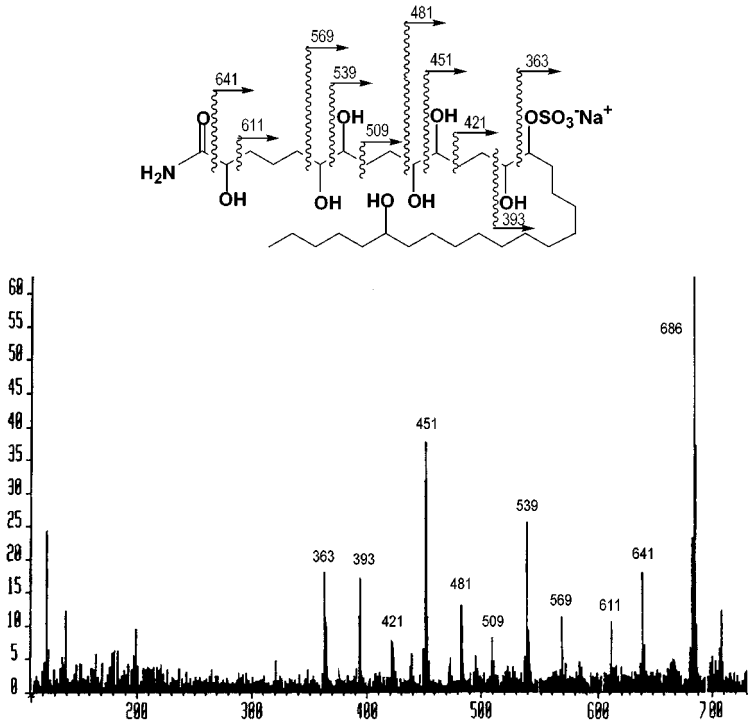



**Figure 1.** Negative ion FAB-MS/MS of turbinamide (**1**).

turbinamide was obviously the position of this function along the aliphatic chain. $^{1}H-^{1}H$ COSY spectrum showed H27−H26/H28 and H30−H32 connectivity patterns. As observed in the HOHAHA spectrum, H27 ($\delta$ 3.52) was correlated with signals at $\delta$ 1.39 ($H26_a/H28_a$), 1.45 ($H26_b/H28_b$), and 0.94 (H32); a further correlation was also observed with a large signal at $\delta$ 1.32−1.36 due to a number of overlapping methylene signals, which hampered determining the number of methylene units separating the hydroxymethine from the terminal methyl group.

Fortunately, a better dispersion of the signals observed in the $^{13}C$ NMR spectrum of turbinamide allowed the placement of the hydroxymethine moiety at C27 on the basis of the following arguments. The HMQC experiment permitted the assignment of the methylene carbon signal at $\delta$ 38.40 to C28, while the $^{13}C$ NMR signals at $\delta$ 33.08 and 23.53 were assigned to C30 and C31, respectively, on the basis of their long-range correlations with the methyl protons at $\delta$ 0.94. Conclusive information was obtained from the HMQC−HOHAHA spectrum, where only four methylene carbon signals, namely, those resonating at $\delta$ 38.40, 26.60, 33.08, and 23.53, were correlated with the terminal methyl protons (see Figure 2). The observed $^{13}C$ NMR chemical shift for C28, C29, C30, and C31 were also in excellent agreement with those calculated using the additivity relationship and the shift parameter reported for alkyl chain.[5] The above data allowed us to define unambiguously the C26−C32 unit; identification of the C1−C16 and C26−C32 fragments hence allowed us to establish the number (nine) of methylene groups between C16 and C26.


**Figure 2.**

Turbinamide (**1**) was evaluated for its cytotoxicity against J774 (murine monocytes/macrophages), C6 (rat glioma), and SH-SY5Y (human neuroblastoma) cell lines. The cell viability was assessed through an MTT conversion assay,[6] and the results are reported in Figure 3.

These preliminary data indicated that turbinamide is greatly more potent in inhibiting C6 (rat glioma) and SH-SY5Y

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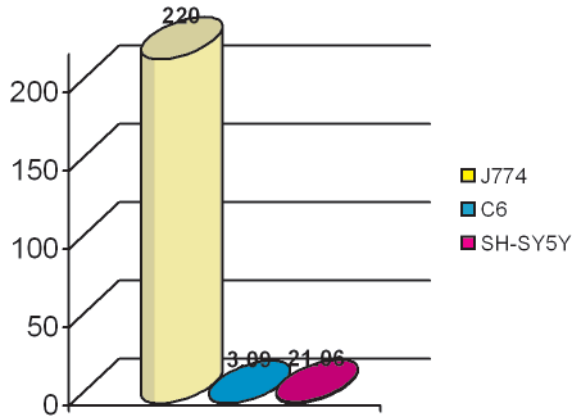


**Figure 3.** In vitro antiproliferative activity ($IC_{50}$, $\mu$g/mL) of turbinamide (**1**) on J774 (rat monocyte/macrophage), C6 (rat glioma), and SH−SY5Y (human neuroblastoma) cell lines. Results are expressed as mean ± SEM of three separate experiments in triplicate.

(human neuroblastoma) rather than J774 (rat monocyte/macrophage) cell proliferation. In particular, we demonstrated that C6 rat glioma cells are very sensible to turbinamide. Further studies, aiming to investigate a possible mechanism responsible of this selective cytotoxic action against nervous system cells, are actually in progress.

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**Supporting Information Available:** IR, $^{1}$H NMR, $^{13}$C NMR, $^{13}$C-DEPT, COSY, HOHAHA, HMBC, and HMQC−HOHAHA spectra of turbinamide **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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