Cytotoxic Bastadin 24 from the Australian Sponge *Ianthella quadrangulata*[⊥]

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A new cytotoxic bastadin, bastadin 24 (**1**), and the previously reported bastadins 4, 5, 6, 7, 12, 13, and 21 (**2**−**8**) were isolated from a polar extract of the Australian marine sponge *Ianthella quadrangulata*. The planar structure of bastadin 24 (**1**) was elucidated as the 25-hydroxy derivative of bastadin 6 (**4**) by employing spectroscopic techniques (NMR, MS, UV, and IR). All isolated bastadins were evaluated for their cytotoxicity toward a panel of 36 human tumor cell lines and were found to be moderately cytotoxic. Bastadin 24 (**1**) exhibited selective cytotoxic activity toward five of the 36 investigated tumor cell lines. Bastadins 7 (**5**) and 12 (**6**) significantly inhibited the serum + hEGF-induced (human epithelial growth factor) tubular formation of human umbilical vein endothelial cells (HUVEC) at a concentration of 1 μg/mL.

The search for new anticancer drugs has led to the discovery of numerous cytotoxic metabolites isolated from marine sponges, such as halichondrin B and hemiasterlin. Synthetic derivatives (E7389 and E7974, respectively) of these sponge-derived natural products are currently in clinical trials for the treatment of cancer.[1,2]

Sponges of the species *Ianthella basta* (family Ianthellidae Hyatt, 1875) have been shown to contain brominated macrocyclic alkaloids, named bastadins, that exhibit cytotoxic activity against cancer cells.[3–7] Moreover, bastadins have recently been proven to inhibit tumor angiogenesis by inducing selective apoptosis to endothelial cells.[8,9] Bastadin 6 displayed significant inhibitory activity on tumor growth in a first *in vivo* xenograft model without any acute toxicity, suggesting that bastadins have the potential to be anticancer agent candidates.[8] Bastadins represent a unique structural class of natural products, composed of two tyrosine-derived dipeptides each linked through ether bonds. In the present study, chemical investigations of a less well investigated *Ianthella* species, *I. quadrangulata*,[10] yielded the new bastadin 24 (**1**), along with the previously reported bastadins 4, 5, 6, 7, 12, 13, and 21 (**2**−**8**),[3–7] as well as noncytotoxic iantherans that possess agonist activity at $P2Y_{11}$ receptors.[11] Then all the isolated bastadins were evaluated for their cytotoxicity toward a panel of 36 human tumor cell lines and were found to be moderately cytotoxic. Bastadin 24 (**1**) showed concentration-dependent inhibition of *in vitro* tumor cell proliferation with a mean IC_{50} value of 1.8 μg/mL combined with significant *in vitro* tumor cell selectivity toward five of the 36 tested tumor cell lines, which indicates 14% selectivity. Moreover, the isolated bastadins (**1**−**8**) were examined for their *in vitro* effects on angiogenesis in human umbilical vein endothelial cells (HUVEC) at different concentrations.

Results and Discussion

Repeated reversed-phase chromatography of the polar extract of the Australian marine sponge *I. quadrangulata* yielded bastadin 24 (**1**) together with the known bastadins 4 (**2**), 5 (**3**), 6 (**4**), 7 (**5**), 12 (**6**, formerly bastadin 9), 13 (**7**, formerly bastadin 12), and 21 (**8**).[3–7]

Optically active bastadin 24 (**1**) was obtained as a white, amorphous solid for which the negative ESIMS spectrum contained a $(M - H)^-$ mass cluster at *m/z* 1107, 1109, 1111, 1113, 1115, 1117, and 1119 corresponding to the presence of six bromine atoms. On the basis of accurate mass measurement [HR-FT/ICR, *m/z* 1106.6694 $(M - H)^-$, Δ −3.3 mmu] the molecular formula of **1** was determined to be $C_{34}H_{26}Br_6N_4O_9$, which is not isomeric with any of the known bastadin alkaloids, but possesses one more oxygen atom than bastadin 6 (**4**), suggesting **1** to be a hydroxy derivative of bastadin 6 (**4**). Inspection of the ^1H NMR spectrum and $^1H-^1H$ COSY NMR data revealed that **1** is composed of four 1,2,3,5-tetrasubstituted aromatic rings, two of which are symmetrically (rings A and C) (2H singlet each, H-8/H-12 and H-27/H-31) and two asymmetrically (rings B and D) (two meta-coupled doublets each, H-17 and H-19; H-36 and H-38) substituted. One of the two-proton singlets (H-27/H-31, δ 7.63, br s) could only be observed in the ^1H NMR spectrum recorded in CD_3CN at 50 °C, but not at room temperature due to the presence of conformers. From the $^1H-^1H$-COSY NMR experiment two $-CH_2CH_2NH-$ spin systems were identified. The remaining proton resonances were ascribed to two geminally coupled protons for a methylene group (H_2-1, δ 3.57 and 3.63, 12.8 Hz) and a singlet (H-25, δ 6.17) for an aliphatic methine group substituted with a hydroxyl group. Two-dimensional $^1H-^{13}C$ HSQC and HMBC experiments (latter optimized for J_{CH} = 8 Hz) allowed two partial structures of **1** to be developed, namely, the northern and southern hemispheres (including rings A and D and rings B and C, respectively; see Table 1). The positions of the bromine and oxygen substituents were assigned making use of the ^{13}C NMR chemical shifts of the respective carbon atoms.

Taking into account the above-discussed results and the calculated 21 degrees of unsaturation, compound **1** had to be a macrocyclic, 2-fold phenyl ether-linked bastadin. The positions of the phenol and ether functionalities of rings B and D were determined on the basis of the $^3J_{C-H}$ couplings between the exchangeable phenolic protons OH-15 and OH-34, respectively, and their ortho-brominated carbons C-16 and C-35, respectively.

This left two possibilities to combine the northern and southern parts of the molecule, i.e., either rings A and C, and B and D, or rings A and B, and C and D are connected via ether bonds. EIMS fragmentation of **1** gave rise to a prominent fragment ion cluster at *m/z* 487, 489, 491, and 493, which is proposed to have the molecular formula $[C_{15}H_8Br_3NO_3]^{+\bullet}$ and derive from a McLafferty rearrangement along with charge migration, as shown in Figure 1.[12] This result secured the phenyl ether conjugation between rings C and D

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Table 1. NMR Spectroscopic Data for Bastadin 24 (**1**) and Bastadin 6 (**4**)

atom no.	$\delta^{13}C^{a,b,c}$ (**1**)	δ $^1H^{b,d}$ (mult., *J* in Hz) (**1**)	$^1H-^1H$ COSY^d (**1**)	HMBC^{d,e} (**1**)	δ $^{13}C^{b,c,f}$ (**4**)
1	28.1, CH_2	3.57 (d, 12.8); 3.63 (d, 12.8)	1	2, 3, 36, 37, 38	28.1, CH_2
2	152.3, qC				152.7, qC
3	163.0, qC				163.7, qC
4	NH	6.89 (t, 6.1)	5	3, 5	NH
5	40.0, CH_2	3.34 (m); 3.51(m)	5, 6, NH-4	3, 6, 7	41.0, CH_2
6	34.0, CH_2	2.75 (m)	5	5, 7, 8, 12	34.6, CH_2
7	141.1, qC				141.4, qC
8/12	134.3, CH	7.46 (br s)		6, 8, 9, 10, 11, 12	134.3, CH
9	117.8, qC				118.2, qC
10	146.8, qC				147.4, qC
11	117.8, qC				118.2, qC
14	145.1, qC				145.5, qC
15	142.2, qC				142.8, qC
16	109.7, qC				110.3, qC
17	127.2, CH	7.10 (d, 1.8)	19	14, 15, 16, 19, 20	127.4, CH
18	131.7, qC				131.8, qC
19	112.8, CH	6.25 (d, 1.8)	17	14, 15, 16, 17, 18, 20	112.8, CH
20	34.0, CH_2	2.61 (m); 2.68 (m)	20, 21	17, 18, 19, 21	34.5, CH_2
21	40.5, CH_2	3.23 (m); 3.30 (m)	20, 21, NH-22	18, 20, 23	39.9, CH_2
22	NH	7.38 (t, 5.8)	21	21, 23	NH
23	164.0, qC				164.5, qC
24	153.9, qC				152.0, qC
25	64.2, CH	6.17 (br s)		23, 24, 26, 27, 28, 30, 31	29.7, CH_2
26	142.4, qC				139.0, qC
27/31	130.1, CH	7.63 (br s)^g		28^g, 30^g	134.3, CH
28	118.2, qC				118.2, qC
29	147.7, qC				147.3, qC
30	118.2, qC				118.2, qC
33	145.2, qC				145.6, qC
34	142.0, qC				142.8, qC
35	109.3, qC				110.0, qC
36	127.5, CH	7.14 (d, 1.8)	38	1, 33, 34, 35, 37, 38	127.9, CH
37	130.4, qC				129.7, qC
38	113.9, CH	6.30 (d, 1.8)	36	1, 33, 34, 35, 36, 37	114.1, CH
OH-25		4.47 (br s)			
OH-34		7.41 (br s)		35	
OH-15		7.46 (br s)		16	
N-OH		9.87 (s)			
N-OH		10.15 (br s)			

[a] CD_3CN, 75 MHz. [b] Assignments are based on extensive 1D and 2D NMR measurements (HMBC, HSQC, COSY). [c] Implied multiplicities determined by DEPT. [d] CD_3CN, 500 MHz. [e] Numbers refer to carbon resonances. [f] Acetone-d_6, 75 MHz. [g] Only detected at 50 °C (CD_3CN, 300 MHz).

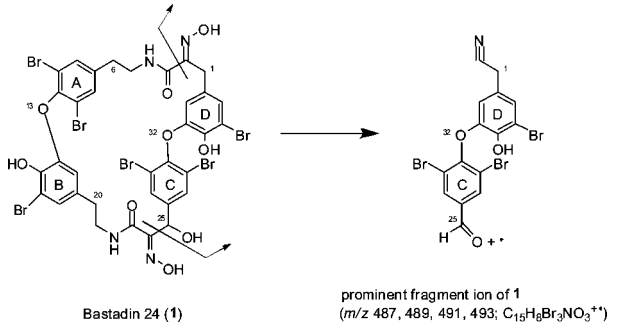


Figure 1. EIMS fragmentation pathway for bastadin 24 (**1**).

and confirmed the position of the benzylic alcohol at C-25 (δ 64.2). Thus, **1** was shown to possess a bastarane skeleton.[3–7]

The stereochemistry of the oxime group at C-2 was assigned on the basis of ^{13}C NMR chemical shifts. Since the α-benzylic carbon C-1 resonated at δ 28.1, the adjacent oxime group must have *E* geometry.[13] In accordance with the data from known bastadins, the double-bond geometry of the second oxime group at C-24 was assumed to be also *E*.[3–7] Finally, for deduction of the absolute stereochemistry at C-25 the Mosher method subsequent to methylation was envisaged.[5] Methylation of **1** with methyl iodide yielded a pentamethylated product, meaning C-25 OH was methylated and thus no longer amenable to Mosher ester derivatization. The fact that **1** is optically active rules out any possibility that the C-25 benzylic hydroxylation resulted from air oxidation during workup.

Table 2. Cytotoxic Activities of **1**–**8**

compound no.	bastadin no.	mean IC_{50} (μg/mL)[a]
1	24	1.8
2	4	2.9
3	5	2.2
4	6	0.7
5	7	3.2
6	12	1.1
7	13	2.4
8	21	8.7

[a] Mean IC_{50} values resulting from 36 different human tumor cell lines.

Hence, compound **1** was defined unambiguously as the 25-hydroxy derivative of bastadin 6 (**4**), and the name bastadin 24 is proposed for **1**.

The structures of bastadins 4–7 (**2**–**5**),[3] 12 (**6**, formerly bastadin 9),[4,5] 13 (**7**, formerly bastadin 12),[6] and 21 (**8**)[7] were identified by comparing their spectroscopic data, and in the case of bastadin 12 (**6**) the optical rotation of its tetramethyl derivative, with published values. All structures were confirmed by interpretation of extensive 1D and 2D NMR measurements (HMBC, HSQC, COSY).

The cytotoxicity of **1**–**8** was determined in a monolayer cell survival and proliferation assay in a panel of 36 human tumor cell lines, comprising 14 different solid tumor types. All examined bastadins (**1**–**8**) proved to be active, exhibiting mean IC_{50} values ranging from 0.7 to 8.7 μg/mL (see Table 2). Bastadin 24 (**1**) showed concentration-dependent inhibition of *in vitro* tumor cell proliferation with a mean IC_{50} value of 1.8 μg/mL combined with

significant *in vitro* tumor cell selectivity toward five of the 36 tested tumor cell lines, which indicates 14% selectivity (using an individual IC_{50} value <1/3 of the mean IC_{50} value as a threshold for above average sensitivity; see Table S1, Supporting Information). These five above average sensitive cell lines were CNXF SF268 (glioblastoma, $IC_{50} = 0.38$ μg/mL), LXFA 629L (lung adenocarcinoma, $IC_{50} = 0.37$ μg/mL), MAXF 401NL (mammary cancer, $IC_{50} = 0.55$ μg/mL), MEXF 276L (melanoma, $IC_{50} = 0.59$ μg/mL), and PRXF 22RV1 (prostate cancer, $IC_{50} = 0.46$ μg/mL). Interestingly its observed cytotoxic selectivity pattern did not correlate with those of any of the standard cytotoxic compounds with known mechanisms of action as deduced by COMPARE analyses. The correlation coefficients were below the significant threshold level of $\rho = 0.6$ in each case. Thus, bastadin 24 (**1**) was identified to be COMPARE-negative, indicating that its mechanism of action was not represented by the standard agents used in this COMPARE analysis.

These results are in good agreement with the reported cytotoxic activities of the bastadin alkaloids.[4,5] Here we describe a significant cytotoxic selectivity of the newly identified bastadin 24 (**1**). Moreover, the bastadin alkaloids have recently attracted attention due to their remarkable tumor angiogenesis inhibitory properties, supporting their potential as anticancer agents.[8,9] Therefore, we evaluated the isolated bastadins (**1**−**8**) for their *in vitro* effects on angiogenesis in human umbilical vein endothelial cells (HUVEC) at different concentrations (0.01, 0.03, 0.1, 0.3, and 1 μg/mL). Bastadins 7 (**5**) and 12 (**6**) significantly inhibited the serum + hEGF-induced (human epithelial growth factor) tubular formation of HUVEC cells at a concentration of 1 μg/mL compared to the control experiments (37.3% and 27.6% inhibition, respectively). Lower concentrations of bastadins 7 (**5**) and 12 (**6**) showed no effects. All the other examined bastadins were not active in this angiogenesis inhibition test system. In contrast to the literature, bastadin 6 (**4**) did not display any dose-dependent inhibition of the formation of a tubular structure, maybe referring to the different growth factors used by Aoki and co-workers (vascular endothelial growth factor VEGF and basic fibroblast growth factor bFGF).[8]

Experimental Section

General Experimental Procedures. Optical rotation was measured on a JASCO DIP 140 polarimeter. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. ^{1}H, ^{13}C, COSY, ROESY, HSQC, and HMBC NMR spectra were recorded in CD_3CN (bastadin 24), CD_3OD (bastadin 5, 12, 21), acetone-d_6 (bastadin 6, 7, 13), or THF-d_8 (bastadin 4) using either a Bruker Avance 300 DPX or 500 DRX spectrometer operating at 300 or 500 MHz for proton and at 75 or 125 MHz for ^{13}C, respectively. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 1.93/117.7 (CD_3CN), 3.35/49.0 (CD_3OD), 2.04/29.8 (acetone-d_6), and 1.85/26.5 (THF-d_8). HPLC-ESIMS measurements were performed employing an Agilent 1100 Series HPLC including DAD, with a RP (reversed-phase) C18 column (Macherey-Nagel Nucleodur 100, 125 mm × 2 mm, 5 μm) and gradient elution (0.25 mL/min, NH_4Ac buffer 2 mmol, from MeOH−H_2O, 10:90, in 20 min to 100% MeOH, then isocratic for 10 min), coupled with an API 2000, Triple Quadrupole LC/MS/MS, Applied Biosystems/MDS Sciex, and ESI source. LREIMS were recorded on a Finnigan MAT 95 spectrometer, and HRESIFT/ICRMS on a Bruker Daltonics APEX-III FT-ICR-MS spectrometer. HPLC was carried out either using a Waters M-6000A pump and a Knauer 298.00 differential refractometer as detector or using a Waters 600 E pump in combination with a Waters 600 controller and a Waters 996 photodiode array detector.

Animal Material. *Ianthella quadrangulata* was collected from Heron Island's, Wistari Reef, Australia, at a depth of 12 m and stored frozen at −20 °C until extraction. A voucher specimen is deposited at the Institute for Pharmaceutical Biology, University of Bonn, voucher number Her 20.

Extraction and Isolation. Thawed sponge tissue (82.5 g dry wt) was extracted with MeOH and a mixture of EtOH and H_2O to yield 31.2 g of brown crude extract, which was fractionated employing VLC (vacuum liquid chromatography) over RP material (Macherey-Nagel, Polygoprep 60–50 C18) using gradient elution from H_2O containing increasing proportions of MeOH, followed by CH_2Cl_2, to yield 10 fractions. ^{1}H NMR investigations of these fractions indicated fractions 7 and 8 to be of further interest, based on the presence of resonances attributable to aromatic protons. Fractions 7 and 8 were further fractionated by RP HPLC (column: Knauer C18 Eurospher-100, 250 × 8 mm, 5 μm) utilizing MeOH−H_2O, 65:35 (2.0 mL/min), to afford bastadins 5 (**3**, 40.0 mg), 6 (**4**, 35.5 mg), 12 (**6**, 28.2 mg), 13 (**7**, 11.6 mg), and 21 (**8**, 29.4 mg) as amorphous solids and a mixture of bastadins 4, 7, and 24 (34.3 mg). Required purification of the mixture was achieved by RP HPLC (column: Macherey-Nagel Nucleodur Sphinx RP, 250 × 4.6 mm, 5 μm; MeOH−H_2O, 67:33, 1.0 mL/min) to give 7.7 mg of bastadin 24 (**1**), 4.1 mg of bastadin 4 (**2**), and 4.0 mg of bastadin 7 (**5**).

Bastadin 24 (1): white, amorphous solid (7.7 mg, 0.009%); $[\alpha]_D^{23}$ –36 (*c* 0.88, MeOH); UV (MeOH) λ_{max} 207 nm (ϵ 109 225) and 280 nm (ϵ 4873); IR (ATR) ν_{max} 3278, 2926, 1703, 1657, 1535, 1498, 1453, 1422, 1360, 1280, 1246, 1225, 1178, 984, 742 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 1); EIMS *m*/*z* 493 (34), 491 (100), 489 (98), 487 (32), 411 (33), 409 (51), 407 (25), 281 (20), 279 (36), 277 (18); HRMS (FT/ICR) calcd for $C_{34}H_{25}{}^{79}Br_6N_4O_9$ $(M - H)^-$ *m*/*z* 1106.6727, found *m*/*z* 1106.6694.

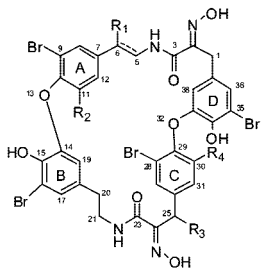
Bastadins 4, 5, 6, 7, 12, 13, and 21 (2–8): (4.1, 40.0, 35.5, 4.0, 28.2, 11.6, and 29.4 mg, respectively); NMR data, and in the case of bastadin 12 the optical rotation of its tetramethyl derivative ($[\alpha]_D$ +3 (*c* 0.05, CH_2Cl_2); lit.: +2.7 (*c* 0.77, CH_2Cl_2),[4] +4.5 (*c* 0.65, CH_2Cl_2)[5]), match the previously published data.[3–7] ^{13}C NMR data of bastadin 6 (**4**), see Table 1.

Methylation of bastadins 12 (6) and 24 (1). Bastadin 12 (12 mg) was stirred with MeI (1.1 mL) and anhydrous K_2CO_3 (0.6 g) in DMF (10 mL) at room temperature for 19 h. The solvent was removed under vacuum to give a yellow residue, which was suspended in CH_2Cl_2 and filtered. Purification of the CH_2Cl_2-soluble material on normal-phase (column: Knauer, Eurospher 100 Si, 5 μm, 250 × 4 mm; 1.0 mL/min) using CH_2Cl_2−EtOAc (95:5) as the solvent system gave 0.7 mg of the tetramethyl derivative of bastadin 12. Bastadin 24 (4.2 mg) was methylated by the same procedure (0.4 mL of MeI, 0.2 g of K_2CO_3, 3 mL of DMF), but the CH_2Cl_2-soluble reaction product contained exclusively the pentamethylated derivative of bastadin 24, analyzed via HPLC ESIMS.

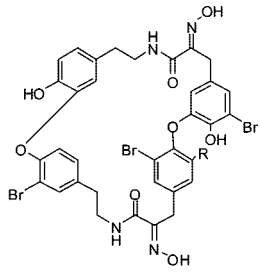
Cytotoxicity Testing. A modified propidium iodide monolayer assay was used to determine the cytotoxic activity of the compounds against human tumor cell lines. The test procedure has been described elsewhere.[14] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice or obtained from the American Type Culture Collection, Rockville, MD, National Cancer Institute, Bethesda, MD, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Briefly, human tumor cell lines were grown at 37 °C in a humidified atmosphere (95% air, 5% CO_2) in monolayer cultures in RPMI 1640 medium supplemented with 10% FCS and phenol red (PAA, Cölbe, Germany). Cells were trypsinized and maintained weekly. Cells were harvested from exponentially growing cultures by trypsination, counted, and plated in 96-well flat-bottomed microplates (140 μL cell suspension, 5×10^3 to 10×10^3 cells/well). After a 24 h recovery to allow cells to resume exponential growth, 10 μL of culture medium (6 control wells per plate) or medium containing the test drug was added to the wells. Each drug concentration was plated in triplicate. After 4 days of incubation the culture medium was replaced by fresh medium containing 6 μg/mL propidium iodide. Microplates were then kept at –18 °C for 24 h, to give a total cell kill. After thawing of the plates, fluorescence was measured using the Cytofluor 4000 microplate reader (Perseptive Biosystems) (excitation 530 nm, emission 620 nm). The amount of viable cells was proportional to the fluorescence intensity. Cytotoxicity including the induction of apoptosis and the inhibition of cell proliferation was recorded as a reduction of the viable cell number relative to control wells and expressed as T/C (test/control) value.

Data Evaluation, Mean Graph Analysis, and COMPARE Analysis. Antiproliferative efficacies of test compounds were described by inhibitory concentrations (IC_{50} values), reflecting concentration-dependent cytotoxicity. Extrapolated IC_{50} values were given if the exact value could not be determined within the test range, and if linear regression of existing T/C values resulted in IC_{50} values within a range of 3-fold the highest test concentration. In the case of resistant cell lines, exhibiting no activities, IC_{50} values were expressed to be greater

Chart 1. Bastadins with the Bastarane Skeleton (a) and the Isobastarane Skeleton (b)



(a)



(b)

Cmpd. No.	Bast. No.	C5–C6 bond	R_1	R_2	R_3	R_4
1	24		H	Br	OH	Br
2	4	$\Delta^{5,6}$	H	H	H	Br
3	5		H	H	H	Br
4	6		H	Br	H	Br
5	7	$\Delta^{5,6}$	H	H	H	H
6	12		OH	Br	H	H

Cmpd. No.	Bast. No.	R
7	13	Br
8	21	H

than the highest test concentration. Cytotoxic selectivity patterns were obtained by mean graph analyses, where the distribution of IC_{50} values obtained for a test compound in the individual tumor types was given in relation to the mean IC_{50} value, obtained for all tumors tested. The individual IC_{50} values were expressed as bars on a logarithmically scaled axis. Bars to the left demonstrated IC_{50} values lower than the mean value (indicating more sensitive tumor models); bars to the right demonstrated higher values (indicating rather resistant tumor models).

The COMPARE algorithm uses *in vitro* activity data to obtain clues as to the mechanism of action of a test compound. The individual IC_{70} values of the test compounds in 36 test cell lines obtained in the monolayer assay were correlated to the corresponding IC_{70} values for more than 100 standard agents determined in exactly these 36 cell lines (see Table S2, Supporting Information). Data for these standard agents are available in the Oncotest database. These standard agents represent the main mechanisms of action of current anticancer drugs. Similarities between the sensitivity pattern of a test compound and those of standard drugs are expressed quantitatively as Spearman correlation coefficients. High correlations ($\rho > 0.6$) between the sensitivity patterns of two compounds (referred to as COMPARE-positive) are indicative of similar mechanisms of action. Low correlations between the sensitivity profile of a test compound on one hand and the profiles of all standard compounds on the other (referred to as COMPARE-negative) indicate that the mechanism of action of the test compound is not represented by the chosen standard compounds.[15]

Tubular Formation Assay (*in Vitro* Angiogenesis Test). ECM-Gel from Engelbreth Hom-Swarm Mouse Sarcoma (Sigma) was diluted by 10% with endothelial cell basal medium containing 2% fetal bovine serum, 0.4% bovine brain extract, 30 μg/mL gentamycin, hEGF, and hydrocortisone (Lonza Group Ltd., Basel, Switzerland) and added to a 96-well plate (40 μL/well). The gel was allowed to solidify for 1 h at 37 °C. Human umbilical vein endothelial cells (HUVEC) were seeded onto the gel (90 μL/well) at a concentration of 6×10^5 cells/mL in the presence or absence of different concentrations of compound (dissolved in 10 μL of medium). Following 24 h of incubation (37 °C, 5% CO_2) the cells were stained with crystal violet (0.5% crystal violet (Sigma) in 50% ethanol/PBS with 5% formaldehyde) for 30 s before washing with PBS. All fluid was removed from the wells and the plate scanned (Epson Perfection 3200). The image was then analyzed using image analysis software (Metamorph, Molecular Devices, Downingtown, PA).

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Supporting Information Available: EIMS, ESIMS, HRMS (FT/ICR), ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of **1**; Table S1 displaying cytotoxicity of **1** in 36 human tumor cell lines in monolayer culture, and Table S2, displaying the standard agents used for the COMPARE analyses. This information is available free of charge via the Internet at http://pubs.acs.org.

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