Cytotoxic Cembranoids from the Soft Corals *Sinularia gibberosa* and *Sarcophyton trocheliophorum*

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Four new cytotoxic cembranoid diterpenes, 11,12-epoxy-1(*E*),3(*E*),7(*E*)-cembratrien-15-ol (**1**), 3,4:11,12-diepoxy-15-methoxy-1(*E*),7(*E*)-cembradiene (**2**), 1(*E*),3(*E*),7(*E*),11(*E*)-cembratetraene-14,15-diol (**3**), and 3,14-epoxy-1(*E*),7(*E*),11(*E*)-cembratriene-4,15-diol (**4**), have been isolated from the soft coral *Sinularia gibberosa*. Selective cytotoxicity was also observed for the new compound, 7β,8α-dihydroxydeepoxysarcophine (**5**), and for sarcophine (**6**), and 16-deoxysarcophine (**7**), isolated from the soft coral *Sarcophyton trocheliophorum*. The structures of the new compounds were determined by spectral and chemical methods.

In our search for bioactive substances from marine organisms, the soft corals *Sinularia gibberosa* Tixier-Durivault (Alcyoniidae) and *Sarcophyton trocheliophorum* Von Marenzeller (Alcyoniidae) were studied because the $CHCl_3$ extracts obtained from them showed significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (murine lymphocytic leukemia) cell cultures as determined by standard procedures.[1] In a previous paper[2] we reported the isolation of a new cytotoxic cembranoid diterpene, sinugibberol (**8**), from *S. gibberosa*. We now report the isolation of four new cytotoxic cembranoid diterpenes, 11,12-epoxy-1(*E*),3(*E*),7(*E*)-cembratrien-15-ol (**1**), 3,4:11,12-diepoxy-15-methoxy-1(*E*),7(*E*)-cembradiene (**2**), 1(*E*),3(*E*),7(*E*),11(*E*)-cembratetraene-14,15-diol (**3**), and 3,14-epoxy-1(*E*),7(*E*),11(*E*)-cembratrien-4,15-diol (**4**), by cytotoxicity-guided fractionation. During the past decade, a number of diterpenes have been found in *S. trocheliophorum*.[3−8] In this investigation, three cytotoxic cembranoids, 7β,8α-dihydroxydeepoxysarcophine (**5**), sarcophine (**6**), and 16-deoxysarcophine (**7**) were obtained from this organism by cytotoxicity-guided fractionation.

Results and Discussion

Compound **1** was isolated as a colorless oil, and its molecular formula was shown to be $C_{20}H_{32}O_2$ by HREIMS. The structure of **1** contained a hydroxy group (IR ν_{max} 3600 cm^{-1}) and a conjugated diene system [UV λ_{max} (log ϵ) 244 (4.0)]. The ^{1}H-NMR spectrum showed the alcohol to be tertiary, a conclusion supported by the fact that the compound resisted acetylation by Ac_2O in pyridine. A six-proton signal appeared to be present at δ 1.36, suggesting that two of the five methyl groups were equivalent and present on a carbon bearing an allylic hydroxyl group. Two other methyl groups were obviously vinylic (δ 1.67, 1.75), and the more downfield signal was placed on the diene system. The final methyl signal was at δ 1.27, and its chemical shift suggested its presence on an epoxide ring. That the remaining oxygen was part of the epoxide ring was indicated by the presence of a doublet of doublets at δ 2.92 (J = 3.2, 9.0 Hz). A one-proton triplet (δ 5.27, 5.3 Hz) was shown to be appropriately positioned for an isolated vinyl methine. The two protons of the diene system appeared as an AB quartet at δ 5.80, 6.27 (J = 10.2 Hz). The ^{13}C-NMR spectrum of compound **1** supported the above assignment, inasmuch as the isopropyl carbinol methyl carbons were coincident at δ 29.7 ppm, and the carbon carrying the oxygen appeared at 73.7 ppm. The epoxide group showed an usual doublet and singlet combination at 60.8 and 61.1 ppm, respectively. The three other methyl quartets were seen at 15.0, 17.5, and 18.0 ppm. The structural features presented above account for the four degrees of unsaturation; a ring was indicated for the fifth degree. Precedents in the soft coral literature,[9] led us to assume the presence of a cembrane ring. From this assumption and the facts that the two protons of the diene system were vicinal and the alcohol function was allylic, the alcohol may be concluded to be allylic to the diene system. Possible structures for the diterpene were quickly reduced to **1** and **9**; compound **9** has been reported previously by Coll *et al*.[10] By comparing the ^{13}C-NMR spectral data of compounds **1** and **10** with compounds **9** and **11**,[10−12] we found similar chemical shift differences among methylene carbons in these two pairs of cembranoids, supporting the assumption that the epoxy group of compound **1** was located at positions C-11, C-12. Assignments of the ^{1}H- and ^{13}C-NMR signals of **1** were made by the application of COSY and HETCOR experiments.[13]

Compound **2** was isolated as colorless prisms, whose molecular formula, $C_{21}H_{34}O_3$ was revealed by HREIMS. ^{1}H-NMR data looked similar to those of **8** except for an additional methyl singlet at δ 3.05. The structure determination of compound **2** was aided by a comparison of the IR and ^{1}H- and ^{13}C-NMR spectral data with those of **8**,[2] since the OH group present in **8** was replaced by a methoxy group in **2** (δ 3.05, 3H, s, OCH_3; 50.3, q, OCH_3). Compound **2** could possibly be an artifact from solvent extraction, but this is unlikely because an attempt to generate **2** by refluxing **8** overnight with MeOH−Me_2CO−$CHCl_3$ (1:1:1) was unsuccessful.

Compound **3** was isolated as a colorless oil. HREIMS showed the molecular formula to be $C_{20}H_{32}O_2$. IR absorptions at 1605 cm^{-1} and 1665 cm^{-1} and UV absorption at 245 nm (log ϵ 3.7) indicated the presence of a conjugated diene. The ^{1}H-NMR spectrum of **3** in $CDCl_3$ indicated the presence of three vinylic methyl groups on quaternary carbons (δ 1.52, 3H, s; 1.67, 3H,

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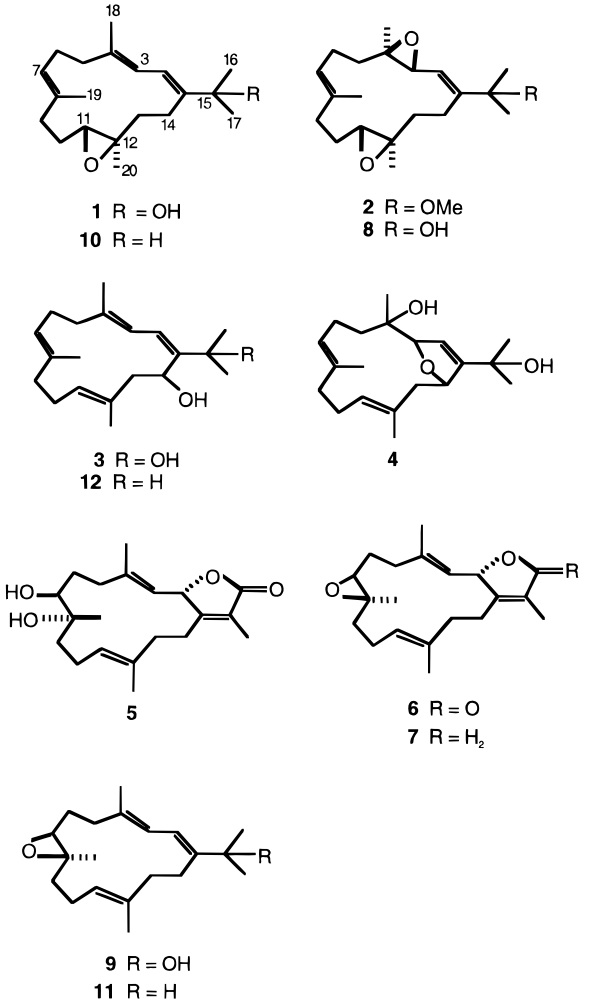
s; 1.74, 3H, s), four olefinic protons (δ 4.91−5.02, 2H, m; 5.85, 1H, d, J = 11.3 Hz; 6.11, 1H, d, J = 11.3 Hz), six isopropyl carbinol methyl protons (δ 1.47, 3H, s; 1.48, 3H, s), and a hydroxy-methine proton (δ 5.00, 1H, m), suggesting the structure of this compound to be quite close to that of sarcophytol-A (**12**).[14] Comparison of ^{1}H- and ^{13}C-NMR spectra of **3**, **1**, and **12** led to the assignment of its structure as **3**.



Compound **4** was isolated as a colorless oil. HREIMS indicated the molecular formula to be $C_{20}H_{32}O_3$. Compound **4** exhibited sharp and well-defined ^{1}H- and ^{13}C-NMR signals. On the basis of the ^{1}H-, ^{13}C-NMR, 2D COSY, and 2D HETCOR spectra, the presence of the following moieties was deduced: two trisubstituted olefins [^{1}H, δ 5.05 (t), 5.23 (dd); ^{13}C, δ 132.7 (s), 130.9 (d), 130.2 (d), 127.5 (d)] bearing two methyls [^{1}H, δ 1.64 (3H, s), 1.56 (3H, s); ^{13}C, δ 17.3 (q), 15.9 (q)], a trisubstituted olefin [^{1}H, δ 5.69 (t); ^{13}C δ 151.9 (s), 122.6 (d)] bearing an isopropyl carbinol [^{1}H, δ 1.47 (3H, s), 1.40 (3H, s); ^{13}C, δ 70.0 (s), 30.5 (q), 30.8 (q)], and a tertiary methyl [^{1}H, δ 0.99 (3H, s); ^{13}C, δ 23.8 (q)]. There were also two oxygenated methine groups [^{1}H, δ 4.96 (dq), 4.66 (dd); ^{13}C, 86.3 (d), 83.1 (d)] and one oxygenated quaternary carbon [^{13}C; δ 74.6 (s)]. In consideration of the molecular formula and number of double bonds, compound **4** was concluded to be bicyclic. Examination of the complex autooxidation products of **3** showed that **4** was the major product, confirming its structural assignment and suggesting that it is could be an oxidative artifact of **3**.

Table 1. Cytotoxicitiy[a] of **1**−**7** (n = 8)

	cell line ED_{50} (μg/mL)			
compd	A549	HT-29	KB	P-388
1	1.03	0.64	0.63	0.01
2	>50	0.36	>50	3.26
3	20.16	0.29	14.92	1.54
4	1.70	1.81	17.12	0.23
5	24.66	22.88	>50	3.27
6	10.38	10.46	>50	2.42
7	15.74	16.07	>50	3.87

[a] For significant activity of pure compounds, an ED_{50} of ≤4.0 μg/mL is required.[1]

The known cembranoid, sarcophine (**6**), previously isolated from *Sarcophyton glaucum*,[15] was obtained as yellow needles (230 mg). Its ^{1}H-NMR, ^{13}C-NMR, and MS were identical with those reported.[15]

Compound **5** was isolated as colorless prisms. HRE-IMS established a molecular formula of $C_{20}H_{30}O_4$; its molecular weight exceeded that of **6** by 32 mass units. IR absorptions at 3480, 1742, 1310, and 1275 cm^{-1} indicated the presence of a hydroxyl group and an α,β-unsaturated-γ-lactone ring. The ^{1}H- and ^{13}C-NMR chemical shifts of **5** were virtually the same as those of **6**, except that the epoxy fuctionality [^{1}H, δ 2.68 (1H, t); ^{13}C, δ 61.9 (d), 60.4 (s)] in **6** had been replaced by two hydroxyl groups [^{1}H, δ 3.59 (1H, d); ^{13}C, δ 73.4 (d), 77.6 (s)] in **5**. Refluxing **6** in 2% H_2SO_4/Me_2CO for 30 min at 60 °C afforded the major product **5**. The physical data of **5** agreed exactly with those of the previously reported 7β,8α-dihydroxydeepoxysarcophine.[16]

Another known cembranoid, 16-deoxysarcophine (**7**), previously isolated from *Sarcophyton* sp.[17] was obtained as colorless prisms (190 mg). Its ^{1}H NMR, ^{13}C NMR, and MS were identical to those reported.[17]

Compounds **1**−**7** exhibited significant cytotoxicity toward the growth of A549, HT-29, KB, and P-388 cells. The cytotoxicity values for compounds **1**−**7** are presented in Table 1.

Experimental Section

General Experimental Procedures. The mp's were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-181 Polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26−30 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Unity Plus 400 NMR spectrometer at 400 MHz and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC.

Animal Materials. The soft corals *Si. gibberosa* and *Sa. trocheliophorum* were collected near Kenting, Taiwan, at a depth 12 m and were stored in a freezer until extraction. The voucher specimens (NSUMR-1031 and NSUMR-1019, respectively) were deposited in the Department of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation. The bodies of the soft coral *Si. gibberosa* (4 kg, wet wt) were sliced and then homogenized with MeOH (3 L × 3), Me_2CO (3 L), and

Table 2. ^{1}H-NMR Data of Compounds **1-5**[a]

	compd				
proton	**1**	**2**	**3**	**4**	**5**
2	6.27 (d,10.2)[b]	5.23 (d,6.8)	6.11 (d,11.3)	5.96 (t,1.2)	5.59 (dd,11.0,1.8)
3	5.80 (d,10.2)	3.33 (d,6.8)	5.85 (d,11.3)	4.96 (dq,1.2,4.8)	4.93 (d,11.0)
7	5.27 (t,5.3)	5.37 (m)	4.95 (m)	5.23 (dd,8.4,4.8)	3.59 (dd,10.4,2.0)
11	2.92 (dd,3.2,9.0)	2.69 (dd,3.1,10.5)	4.95 (m)	5.05 (t,6.8)	4.99 (t,6.0)
14			5.00 (m)	4.66 (dd,5.2,1.2)	
16	1.36 (s)	1.30 (s)	1.47 (s)	1.40 (s)	
17	1.36 (s)	1.30 (s)	1.48 (s)	1.47 (s)	1.81 (s)
18	1.75 (s)	1.27 (s)	1.74 (s)	0.99 (s)	1.92 (s)
19	1.67 (s)	1.67 (s)	1.52 (s)	1.56 (s)	1.20 (s)
20	1.27 (s)	1.30 (s)	1.67 (s)	1.64 (s)	1.66 (s)
$-OCH_3$		3.05 (s)			

[a] Chemical shifts were determined at 400 MHz in $CDCl_3$. The values are in ppm downfield from TMS. [b] *J* values in Hz in parentheses.

then CH_2Cl_2 (3 L × 2). After removal of solvent *in vacuo*, the residue (300 g) was partitioned between $CHCl_3$ and H_2O. Column chromatography of the $CHCl_3$ extract (60 g) was undertaken using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity. Elution by $CHCl_3$−MeOH (9:1) afforded fractions containing cembranoids **1-4**, which were purified by column chromatography over Si gel with *n*-hexane−EtOAc, $CHCl_3$−Me_2CO, or EtOAc−*n*-hexane−$CHCl_3$ as eluting solvents.

The bodies of the soft coral *Sa. trocheliophorum* (2 kg, wet wt) were sliced and then homogenized with MeOH (2 L × 3), Me_2CO (2 L), and then CH_2Cl_2 (2 L × 2). After removal of solvent *in vacuo*, the residue (160 g) was partitioned between $CHCl_3$ and H_2O. Column chromatography of the $CHCl_3$ extract (30 g) was undertaken using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity. Elution by $CHCl_3$−MeOH (19:1) afforded fractions containing cembranoids **5**−**7**. Compound **5** was purified by column chromatography over Sephadex LH-20 with *n*-hexane−CH_2Cl_2 as eluting solvent. Compounds **6** and **7** were purified by column chromatography over Si gel with *n*-hexane−Me_2CO (4:1) and *n*-hexane−EtOAc (6:1) as eluting solvents.

11,12-Epoxy-1(*E*),3(*E*),7(*E*)-cembratrien-15-ol (1): 40 mg; colorless oil; $[\alpha]^{25}_D$ −8.6° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.1), 244 (4.0) nm; IR (CCl_4) ν_{max} 3620, 2972, 1684, 1444, 1386, 1250, 1170, 1101, 934, 878 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS (70 eV) *m*/*z* $[M]^+$ 304 (2), 286 (5), 271 (3), 243 (3), 203 (5), 187 (6), 175 (15), 159 (14), 147 (17), 135 (36), 133 (46), 121 (79), 107(82), 95 (65), 93 (80), 81 (87), 67 (78), 59 (64), 43 (100); HREIMS *m*/*z* 304.2399; calcd for $C_{20}H_{32}O_2$ 304.2394.

3,4:11,12-Bisepoxy-15-methoxy-1(*E*),7(*E*)-cembradiene (2): 8 mg; colorless prisms; mp 98−100 °C; $[\alpha]^{25}_D$ +138.9° (*c* 0.024, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.4) nm; IR (KBr) ν_{max} 2900, 1450, 1375, 1230, 1150, 1060, 910, 870, 850, 820 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m*/*z* $[M]^+$ 334 (5), 302 (25), 261 (14), 241 (6), 193 (26), 151 (39), 125 (52), 109 (69), 73 (100), 55 (35); HREIMS *m*/*z* 334.2509, calcd for $C_{21}H_{34}O_3$ 334.2509.

1(*E*),3(*E*),7(*E*),11(*E*)-Cembratetraene-14,15-diol (3): 30 mg; colorless oil; $[\alpha]^{25}_D$ +59.1° (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.0), 245 (3.7) nm; IR ($CHCl_3$) ν_{max} 3360 (OH), 1665, 1605, 1060 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR: see Table 3; EIMS *m*/*z* $[M]^+$ 304 (1), 286 (3), 271 (3), 257 (2), 243 (4), 122 (36), 135 (46), 43 (100); HREIMS *m*/*z* 304.2392, calcd for $C_{20}H_{32}O_2$ 304.2394.

Table 3. ^{13}C-NMR Data of Compounds **1**−**5**[a]

	compd				
carbon	**1**	**2**	**3**	**4**	**5**
1	146.5 (s)	146.9 (s)	145.2 (s)	151.9 (s)	163.5 (s)
2	118.8 (d)	125.6 (d)	120.6 (d)	122.6 (s)	79.8 (d)
3	120.0 (d)	59.4 (d)	120.4 (d)	83.1 (d)	121.5 (d)
4	133.3 (s)	61.7 (s)	138.2 (s)	74.6 (s)	144.7 (s)
7	127.1 (d)	124.8 (d)	124.5 (d)	127.5 (d)	73.4 (d)
8	138.5 (s)	135.0 (s)	134.3 (s)	132.7 (s)	77.6 (s)
11	60.8 (d)	62.3 (d)	126.4 (s)	130.2 (d)	125.9 (d)
12	61.1 (s)	61.3 (s)	132.6 (s)	130.9 (s)	135.3 (s)
13			49.2 (t)	45.6 (t)	
14			72.3 (d)	86.3 (d)	
15	73.7 (s)	77.3 (s)	75.8 (s)	70.0 (s)	123.4 (s)
16	29.7 (q)	26.4 (q)	34.0 (q)	30.8 (q)	175.6 (s)
17	29.7 (q)	25.0 (q)	31.9 (q)	30.5 (q)	9.5 (q)
18	18.0 (q)	18.0 (q)	17.0 (q)	23.8 (q)	16.9 (q)
19	15.0 (q)	14.7 (q)	15.8 (q)	15.9 (q)	25.3 (q)
20	17.5 (q)	16.0 (q)	17.3 (q)	17.3 (q)	15.9 (q)
CH_2	38.8 (t)	39.9 (t)	39.1 (t)	39.2 (t)	37.6 (t)
	38.0 (t)	38.2 (t)	38.6 (t)	38.7 (t)	37.1 (t)
	36.8 (t)	36.8 (t)	24.9 (t)	25.1 (t)	36.1 (t)
	24.9 (t)	25.0 (t)	24.5 (t)	21.9 (t)	27.5 (t)
	24.3 (t)	24.5 (t)			27.4 (t)
	23.5 (t)	22.5 (t)			24.8 (t)
OCH_3		50.3 (q)			

[a] Chemical shifts were determined at 100.6 MHz in $CDCl_3$. The values are in ppm downfield from TMS.

3,14-Epoxy-1(*E*),7(*E*),11(*E*)-cembratriene-14, 15-diol (4): 12 mg; colorless oil; $[\alpha]^{25}_D$ −10.6° (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (3.9) nm; IR ($CHCl_3$) ν_{max} 3450 (OH), 1080, 960, 950 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m*/*z* $[M]^+$ 320 (3), 302 (14), 300 (2), 295 (2), 284 (3), 262 (4), 147 (11), 136 (12), 109 (100); HREIMS *m*/*z* 320.2343, calcd for $C_{20}H_{32}O_3$ 320.2343.

7*β*,8α-Dihydroxydeepoxysarcophine (5): 24 mg; colorless prisms; mp 143−145 °C; $[\alpha]^{25}_D$ +106° (*c* 0.024, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.1) nm; IR ($CHCl_3$) ν_{max} 3480 (OH), 2940, 1742, 1680, 1480, 1400, 1310, 1275, 1200, 1000, 960, 780 cm^{-1}; ^{1}H-NMR, see Table 2; ^{13}C NMR, see Table 3; EIMS *m*/*z* $[M]^+$ 334 (26), 316 (100), 298 (13), 249 (24), 164 (80), 119 (34), 81 (66), 55 (77); HREIMS *m*/*z* 334.2139, calcd for $C_{20}H_{30}O_4$ 334.2136.

Sarcophine (6): 230 mg; yellow needles; mp 130−132 °C; $[\alpha]^{25}_D$ +96.7° (*c* 0.01, $CHCl_3$).

2% H_2SO_4/Me_2CO treatment of sarcophine (6): Sarcophine (10 mg) was stirred for 30 min in a solution of Me_2CO (5 mL) and aqueous 2% H_2SO_4 (1 mL) at 60 °C to afford **5**.

16-Deoxysarcophine (7): 190 mg; colorless prisms; mp 76−77 °C; $[\alpha]^{25}_D$ +150° (*c* 0.1, MeOH).

Cytotoxicity Testing. To measure the cytotoxic activities of pure compounds or crude fractions against A549, HT-29, KB, and P-388, each cell line was initiated at 750, 750, 2000, and 1500 cells/well, respectively, in 96-well microtiter plates. Three to eight concentrations encompassing an 8- to 128-fold range were evaluated on each cell line. A549, HT-29, KB, and P-388 cells were enumerated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) after the exposure to test samples for 6, 6, 3, and 3 days, respectively. Of 1 mg/mL MTT, 50 μL was added to each well, and plates were incubated at 37 °C for a further 5 h. Supernatant was aspirated with a Dynatech automatic washer. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and the plate was read immediately on a microtiter plate reader (Dynatech) at a wavelength of 540 nm. The ED_{50} value was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cell in the MTT assay.[18]

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