Oxidative Halogenation of Substituted Pyrroles with Cu(II). Part IV. Bromination of 2-(2'-Hydroxybenzoyl)pyrrole. A New Synthesis of Bioactive Analogs of Monodeoxypyoluteorin

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The selective bromination with copper(II) bromide of the pyrrole ring in 2-(2'-hydroxybenzoyl)pyrrole **II** in the heterogeneous phase is described giving in almost quantitative yield the 4,5-dibromo derivative **VI**. The subsequent introduction of halogen into the phenol moiety was observed when the reaction was performed in the homogeneous phase with an excess of halogenating agent. The pentabromo derivative **IX**, a compound very active against *Staphylococcus aureus* (*mic* = 17 nmoles per dm^{-3}), was obtained by exhaustive bromination of the title compound. Poor yields of chloro derivatives of **II** were obtained by reaction of the parent compound with copper(II) chloride.

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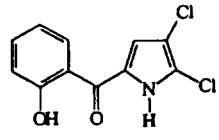
Introduction.

In the last paper of this series [1] we described the oxidative halogenation of 2-benzoylpyrrole with copper(II) halogenides in homogeneous as well as heterogeneous phase, giving in high yields derivatives selectively halogenated at the 4- and 5-positions of the pyrrole ring. The interesting microbiological activity of monodeoxypyoluteorin (**I**), a powerful bactericidal substance which is more active than pyoluteorin itself against *Staphylococcus aureus* [2], as well as the activity of some monodeoxypyoluteorin bromo and chloro analogs [2], prompted us to extend our study to the halogenation of 2-(2'-hydroxybenzoyl)pyrrole in order to ascertain whether copper(II) halogenides could be employed in the direct halogenation of this compound without protecting the phenolic function. This hypothesis finds support in the observation that when hydroxyacetophenones were brominated with copper(II) bromide no products of ring bromination were observed [3]. In the present work the results of our research are reported.

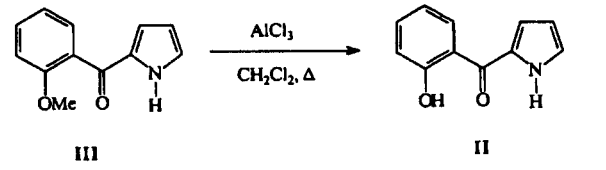


I

Results.

a. Synthetic Aspects.

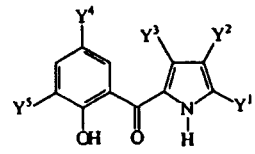
The molecular skeleton of **II** was assembled by allowing pyrrylmagnesium bromide to react with 2-methoxybenzoyl chloride according to the procedure reported by Davies and Hodge in the synthesis of 2-(2',6'-dimethoxybenzoyl)pyrrole [4]. 2-(2'-Methoxybenzoyl)pyrrole (**III**), which was thus obtained in 58% yield, was purified from the 3-isomer by precipitation of its cupric complex from a methanol solution. The subsequent demethylation of compound **III**, using aluminum chloride in anhydrous dichloromethane, afforded the phenol derivative **II** in an 87% yield (Equation 1).



III II 1

Monobromination of **II**, carried out using 1 equivalent of copper(II) bromide at 60° in a heterogeneous phase (1:1 *v/v* chloroform-ethyl acetate mixture), gave rise to the prevailing formation of 4-bromo-2-(2'-hydroxybenzoyl)pyrrole (**V**) together with minor amounts of the 5-isomer **IV** (Table 1, entry 1). Owing to the difficulties in the separation of **V** from the parent pyrrole **II**, the reaction was performed with an excess of halogenating reagent until the complete disappearance of **IV** (Table 1, entry 2). Under these conditions, only **V**, together with the 4,5-dibromo derivative **VI**, was isolated in a 4:1 molar ratio. On the other hand, under the same experimental conditions but using an excess of halogenating reagent, **VI** was obtained in a nearly quantitative yield (Table 1, entry 3).

A somewhat different course of the reaction was observed when the bromination was performed in a homogeneous phase (acetonitrile). In fact, using stoichiometric amounts of halogenating reagent under a mild stream of nitrogen, the dibromination went to completion within 6-8 hours at room temperature (Table 1, entry 4). On the other hand, either using an excess (3 equivalents)



IV Y^1 = Br, Y^2 = Y^3 = Y^4 = Y^5 = H
V Y^2 = Br, Y^1 = Y^3 = Y^4 = Y^5 = H
VI Y^1 = Y^2 = Br, Y^3 = Y^4 = Y^5 = H
VII Y^1 = Y^2 = Y^4 = Br, Y^3 = Y^5 = H
VIII Y^1 = Y^2 = Y^4 = Y^5 = Br, Y^3 = H
IX Y^1 = Y^3 = Y^2 = Y^4 = Y^5 = Br
X Y^1 = Cl, Y^2 = Y^3 = Y^4 = Y^5 = H
XI Y^2 = Cl, Y^1 = Y^3 = Y^4 = Y^5 = H

of copper(II) bromide (Table 1, entry 5) or performing the reaction in the presence of atmospheric oxygen, compound **VI** underwent a further bromination, affording the 4,5,5'-tribromo derivative **VII**. As reported in Table 2, **VII** showed activity towards a *Staphylococcus* sp., which on a molar basis was 6 times higher than that of **VI**. This trend, which is consistent with what was reported by Durham *et al.* [2] about the positive effects of a halogen atom in the 5'- position on the bactericidal activity of these substances, induced us to study the exhaustive bromination with copper(II) bromide of compound **II**. This reaction was carried out at room temperature using a large excess of halogenating reagent (Table 1, entry 7). After 6 days, a pentabromo derivative **IX** was obtained in an almost quantitative yield. Increasing amounts of the tetrahalogenated intermediate **VIII**, which on tlc analysis showed an R_f almost identical, in several eluents, to that of **VII**, were obtained by decreasing the reaction time (Table 1, entry 6). The structures of all these reaction products were assigned on the basis of their spectroscopic data (^{1}H nmr and eims).

Table 1

Halogenation of 2-(2'-Hydroxybenzoyl)pyrrole (**II**) with Copper(II) Halogenides

Entry	CuX_2 / **II** molar ratio	Solvent [a]	Reaction time, hours (*T* /°C)	Products (isolated % yields)
1	2 [b]	A	48 (60)	**IV** (15) **V** (n.d.) [d]
2	3 [b]	A	72 (60)	**V** (72) **VI** (18)
3	6.5 [b]	A	72 (60)	**VI** (95)
4	4 [b,e]	B	6-8 (20)	**VI** (88)
5	6 [b,e]	B	32 (20)	**VI** (30) **VII** (56)
6	10 [b]	B	96 (20)	**VIII** (15) **IX** (68)
7	10 [b]	B	144 (20)	**IX** (88)
8	2 [c]	B	24 (60)	**X** (28) **XI** (n.d.)
9	2 [c]	B	48 (60)	**X** (24) **XI** (10) **I** (n.d.)
10	5 [c]	B	72 (60)	**I** (28)

[a] A: 1/1 (*v/v*) Chloroform-ethyl acetate; B: acetonitrile. [b] X = Br. [c] X = Cl. [d] Not determined. [e] Reaction carried out under nitrogen atmosphere.

Table 2

Antibacterial Activity (*mic*/mg per dm^3)

Compound	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 27853
IV	>50	50	>50
V	>50	>50	>50
VI	>50	3.1	>50
VII	>50	0.62	>50
VIII	>50	0.05	>50
IX	3.1	0.01	>50
Amikacin	10	2	5

The chlorination of the same substrate with copper(II) chloride proceeded slowly and only at temperatures above 60° (Table 1, entry 8), as already observed in the case of 2-benzoylpyrrole [1]. Tlc showed the initial formation of the 5-chloro derivative **X** as the major product accompanied by a little amount of the 4-chloro isomer **XI**. Unfortunately, separation of this latter compound from the educt by column chromatography proved extremely tedious because of the very similar retentions of the two compounds. In order to obtain a sample of compound **XI** free from the parent compound **II**, the reaction was carried out until the appearance of appreciable amounts of the dichloro derivative **I** (*ca.* 48 hours) (Table 1, entry 9). This latter compound was obtained virtually free from monohalogenation products by allowing the educt to react with an excess of copper(II) chloride for 72 hours (Table 1, entry 10). However, the yield of the reaction was lower than that found in the case of 2-benzoylpyrrole [1], likely due to the higher sensitivity of the phenol moiety to side reactions such as oxidation.

b. Bioactivity Assays.

The results of antibacterial screening, expressed as minimum inhibitory concentration (*mic*), are reported in Table 2. All the compounds showed no antifungal activity at the highest concentration tested (50 mg per dm^3). Except for compound **IX**, no activity was observed against gram-negative strains (*E. coli* and *P. aeruginosa*). In contrast, all the tested compounds, except compound **V**, were active against *S. aureus*. For compound **IX**, which showed the best *mic*, we also determined the minimum bactericidal concentration (*mbc*) *versus S. aureus*, whose value was 0.32 mg per dm^3. Finally, compounds **VIII** and **IX** were also tested for preliminary cytotoxicity assay towards mammalian cells. In particular, we determined the inhibition of proliferation of Swiss mouse embryos fibroblasts (3T6 cells) as a cytotoxicity test (Table 3).

Conclusions.

The method of bromination just described afforded the 4,5-dibromo derivative **VI** in a nearly quantitative yield.

Table 3

Cytotoxicity *vs.* Swiss Mouse Embryos Fibroblasts (3T6 cells)

Compound	ic_{50}/mg per dm^3
VIII	2
IX	5

Under an excess of halogenating reagent and operating in a homogeneous phase, the further bromination derivatives **VII, VIII** and **IX**, all not previously reported in the literature, were obtained. We also found that the antibacterial activities of these compounds towards *S. aureus* increase with the number of bromine atoms. A similar trend of activity has been already reported in the literature for the chloro analogs of **VI** (*viz.* compound **I**) and **VII** [2] as well as those of **VIII** and **IX** (Pyrrolomycins C and D, respectively, both produced by fermentation with *Streptomyces* spp.) [5]. In particular, compound **IX** proved very active towards *S. aureus* (*mic* = 17 nmoles per dm^3) being, on a molar basis, about 200 times more potent than the well known antibiotic Amikacin. It is worthy of mention that compound **IX**, in spite of its cytotoxicity, showed an interesting selectivity index (*i.e.* the ratio of ic_{50} to *mic*) towards eukaryotic cells equal to 500.

EXPERIMENTAL

All melting points were determined on a Buechi-Tottoli micro melting point apparatus. The ir spectra were recorded at room temperature in Nujol mulls with a Perkin Elmer Infrared 137 E spectrometer. The ^1H nmr spectra were recorded at room temperature on a Bruker SF 250 spectrometer in DMSO-d_6, unless otherwise specified, using tetramethylsilane as the internal standard. Eims were recorded on a Jeol JMS-01-SG-2 spectrometer (75 eV). Elemental analyses were performed on a Hewlett Packard 185 B CHN analyzer. Chromatographic separations were carried out on columns packed with Kieselgel 60 from Merck (70-230 mesh ASTM). All reactions were monitored by tlc on 0.2 mm silica gel 60 F254 (Merck) plates using uv light for visualization.

All the compounds were tested for their *in vitro* growth inhibitory activity against the following bacteria and yeasts: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 2091, *Candida tropicalis* ATCC 13803. Amikacin (Sigma) was used as growth inhibitory standard and all experiments were performed at least in duplicate.

2-(2'-Methoxybenzoyl)pyrrole (**III**).

To a stirred suspension of pyrrylmagnesium bromide, which was obtained from 2.4 g (100 mmoles) of magnesium turnings, 15 g (140 mmoles) of bromoethane and 6.7 g (100 mmoles) of pyrrole, in 200 ml of anhydrous diethyl ether, 17 g (100 mmoles) of 2-methoxybenzoyl chloride were added dropwise under nitrogen atmosphere. The reaction mixture was heated at reflux for 0.5 hour, then concentrated at reduced pressure, and the residue was quenched with cold 10% sulfuric acid. After stirring for 2 hours, the solid was filtered, then washed with water and dissolved in 50 ml of methanol. Copper(II) acetate (20 g, 100 mmoles) was added to the solution. The cupric complex was filtered, washed with a little methanol and decomposed with 20% sulfuric acid. The crude 2-(2'-methoxybenzoyl)pyrrole was extracted with diethyl ether and the ethereal extracts were evaporated to afford 11.6 g (58%) of the title compound, mp 132° (lit [2] 130-132°). The spectral properties of this compound were in good agreement with those previously reported [2].

2-(2'-Hydroxybenzoyl)pyrrole (**II**).

A solution of 10 g (50 mmoles) of 2-(2'-methoxybenzoyl)pyrrole (**III**), 15 g (110 mmoles) of aluminum chloride in 100 ml of dry dichloromethane was heated at reflux. After 4 hours, the mixture was evaporated. The residue was decomposed with ice/water, then 100 ml of 10% sulfuric acid and 200 ml of diethyl ether were added, and the mixture was vigorously stirred until all the solid residual was dissolved. The ethereal layer was separated and the aqueous phase extracted with ether (2 x 100 ml). The combined extracts were washed with water until neutrality, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by chromatography using 95:5 (*v/v*) cyclohexane-ethyl acetate mixture as eluent to give 2-(2'-hydroxybenzoyl)pyrrole (8.1 g, 87% yield) as yellow needles, mp 80° (from petroleum ether 40-60°) (lit [2] 74°). The spectral properties of this compound were in good agreement with those previously reported [2].

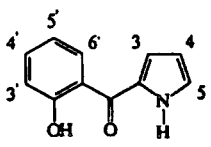
General Procedure for the Halogenation of 2-(2'-Hydroxybenzoyl)pyrrole (**II**) (Table 1).

In a typical experiment, 1.87 g (10.0 mmoles) of compound **II** in a 1:1 (*v/v*) chloroform-ethyl acetate mixture (100 ml) or in acetonitrile (50 ml) were stirred with solid copper(II) halogenide at the temperature reported in Table 1. This Table also shows the copper halogenide/compound **II** molar ratios employed. The reaction mixture was stirred for the required length of time in an open vessel, unless otherwise specified (Table 1), then diluted with 200 ml of diethyl ether and quenched with 10% sulfuric acid (100 ml) and 1 mole per dm^3 iron(III) chloride solution (50 ml). When all of the cuprous salt had been dissolved, the mixture was extracted with diethyl ether (3 x 100 ml), the organic phase was separated, washed with water until neutrality, dried over anhydrous sodium sulfate and evaporated. The isolated products were purified by column chromatography using proper petroleum ether (40-60°)-ethyl acetate mixtures as eluents.

5-Bromo-2-(2'-hydroxybenzoyl)pyrrole (**IV**).

This compound was recrystallized from ethanol, mp 139-140°; ^1H nmr (deuteriochloroform): δ 6.35 (dd, 1H, J = 4.0 and 2.5 Hz, after treatment with deuterium oxide d, J = 4.0 Hz, H-4), 6.94 (br t, 1H, J = 7.8 Hz, H-5'), 6.97 (br d, 1H, J = 4 Hz, H-3), 7.01 (br d, 1H, J = 7.8 Hz, H-3'), 7.49 (dt, 1H, J = 7.8 and 1.4 Hz, H-4'), 7.97 (dd, 1H, J = 7.8 and 1.4 Hz, H-6'), 10.5 (s, 1H, H-1), 11.5 (s, 1H, OH); eims: m/z 265, 267 (M^+); ir: ν 3265 (NH), 1620 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_8BrNO_2$: C, 49.65; H, 3.03; N, 5.26. Found: C, 49.51; H, 2.94, N, 5.02.



II

4-Bromo-2-(2'-hydroxybenzoyl)pyrrole (V).

This compound was recrystallized from ethanol, mp 169°; ^{1}H nmr: δ 6.79 (br s, 1H, H-3), 6.94 (br t, 1H, J = 7.9 Hz, H-5'), 6.97 (br d, 1H, J = 7.9 Hz, H-3'), 7.37 (br s, 1H, H-5), 7.43 (br t, 1H, J = 7.9 Hz, H-4'), 7.59 (dd, 1H, J = 7.9 and 1.0 Hz, H-6'), 10.61 (s, 1H, OH), 12.43 (s, 1H, H-1); eims: m/z 265, 267 (M^+); ir: ν 3300 (NH), 1630 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_8BrNO_2$: C, 49.65; H, 3.03; N, 5.26. Found: C, 49.70; H, 3.18; N, 5.12.

4,5-Dibromo-2-(2'-hydroxybenzoyl)pyrrole (VI).

This compound was recrystallized from ethanol, mp 153-154° (lit [2] 151-152°); ^{1}H nmr: δ 6.78 (s, 1H, H-3), 6.93 (br t, 1H, J = 7.8 Hz, H-5'), 6.97 (br d, 1H, J = 7.8 Hz, H-3'), 7.42 (dt, 1H, J = 7.8 and 1.6 Hz, H-4'), 7.51 (br d, 1H, J = 7.8 Hz, H-6'), 10.4 (s, 1H, OH), 13.3 (s, 1H, H-1); eims: m/z 343, 345, 347 (M^+); ir: ν 3260 (NH), 1620 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_7Br_2NO_2$: C, 38.30; H, 2.05; N, 4.06. Found: C, 38.51; H, 2.15; N, 3.98.

4,5,5'-Tribromo-2-(2'-hydroxybenzoyl)pyrrole (VII).

This compound was recrystallized from ethanol, mp 207-208°; ^{1}H nmr: δ 6.71 (s, 1H, H-3), 6.91 (d, 1H, J = 8.6 Hz, H-3'), 7.47 (d, 1H, J = 2.5 Hz, H-6'), 7.51 (dd, 1H, J = 8.6 and 2.5 Hz, H-4'), 10.3 (s, 1H, OH), 13.3 (s, 1H, H-1); eims: m/z 421, 423, 425, 427 (M^+); ir: ν 3245 (NH), 1620 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_6Br_3NO_2$: C, 31.17; H, 1.43; N, 3.30. Found: C, 31.02; H, 1.36, N, 3.09.

4,5,3',5'-Tetrabromo-2-(2'-hydroxybenzoyl)pyrrole (VIII).

This compound was recrystallized from acetone, mp 219°; ^{1}H nmr: δ 6.87 (s, 1H, H-3), 7.61 (d, 1H, J = 2.2 Hz, H-6'), 7.95 (d, 1H, J = 2.2 Hz, H-4'), 10.6 (s, 1H, OH), 13.4 (s, 1H, H-1); eims: m/z 499, 501, 503, 505, 507 (M^+); ir: ν 3265 (NH), 1610 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_5Br_4NO_2$: C, 26.28; H, 1.00; N, 2.79. Found: C, 26.41; H, 1.13; N, 2.90.

3,4,5,3',5'-Pentabromo-2-(2'-hydroxybenzoyl)pyrrole (IX).

This compound was recrystallized from acetone, mp 225-226°; ^{1}H nmr: δ 7.49 (d, 1H, J = 2.4 Hz, H-6'), 7.89 (d, 1H, J = 2.4 Hz, H-4'), 10.2 (s, 1H, OH), 13.6 (s, 1H, H-1); eims: m/z 577, 579, 581, 583, 585, 587 (M^+); ir: ν 3285 (NH), 1615 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_4Br_5NO_2$: C, 22.71; H, 0.69; N, 2.41. Found: C, 22.92; H, 0.80; N, 2.26.

4-Chloro-2-(2'-hydroxybenzoyl)pyrrole (X).

This compound was recrystallized from ethanol, mp 160-162° (lit [6] 160-162°); ^{1}H nmr (deuteriochloroform): δ 6.96 (dt, 1H, J = 7.9 and 1.6 Hz, H-5'), 6.98 (dd, 1H, J = 3.1 and 1.4 Hz, after treatment with deuterium oxide d, J = 3.1 Hz, H-3), 7.05 (dd, 1H, J = 7.9 and 1.6 Hz, H-3'), 7.11 (dd, 1H, J = 3.1 and 1.4 Hz, after treatment with deuterium oxide d, J = 3.1 Hz, H-5), 7.50 (dt, 1H, J = 7.9 and 1.6 Hz, H-4'), 7.99 (dd, 1H, J = 7.9 and 1.6 Hz, H-6'), 9.67 (s, 1H, H-1), 11.6 (s, 1H, OH); eims: m/z 221 (M^+); ir: ν 3310 (NH), 1630 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; N, 6.32. Found: C, 59.71; H, 3.76; N, 6.19.

5-Chloro-2-(2'-hydroxybenzoyl)pyrrole (XI).

This compound was recrystallized from ethanol, mp 126-127°; ^{1}H nmr (deuteriochloroform): δ 6.25 (br d, 1H, J = 4 Hz, H-4), 7.0 (m, 3H, H-5' + H-3 + H-3'), 7.44 (br t, 1H, J = 8 Hz, H-4'), 7.94 (br d, 1H, J = 8 Hz, H-6'), 10.5 (s, 1H, H-1), 11.5 (s, 1H, OH); eims: m/z 221 (M^+); ir: ν 3260 (NH), 1620 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; N, 6.32. Found: C, 59.51; H, 3.57; N, 6.25.

4,5-Dichloro-2-(2'-hydroxybenzoyl)pyrrole (I).

This compound was recrystallized from ethanol, mp 175-176° (lit [2,6] 174-176°); ^{1}H nmr (deuteriochloroform): δ 6.97 (dt, 1H, J = 7.8 and 1.6 Hz, H-5'), 6.99 (s, 1H, H-3), 7.06 (br d, 1H, J = 8 Hz, H-3'), 7.52 (dt, 1H, J = 7.8 and 1.6 Hz, H-4'), 7.92 (dd, 1H, J = 7.8 and 1.6 Hz, H-6'), 10.3 (s, 1H, H-1) 11.4 (s, 1H, OH); eims: m/z 255 (M^+); ir: ν 3240 (NH), 1625 (CO) cm^{-1}.

Anal. Calcd. for $C_{11}H_7Cl_2NO_2$: C, 51.59; H, 2.76; N, 5.47. Found: C, 51.50; H, 2.71; N, 5.49.

Antibacterial Assay.

A series of solutions of each substance, whose respective concentrations ranged from 0.005 to 50 mg per dm^3 (obtained by twofold serial dilution) was prepared in tryptose phosphate broth (Difco) using a 5 mg per ml solution of each substance in a 1:1 (v/v) DMF/sodium hydroxide mixture (0.1 mole per dm^3). To each series test tube 100 mm^3 of a bacterial suspension, containing 10^6 cfu/ml (colony forming units) [7] was added. The test tubes were incubated at 37° for 24 hours, the minimum inhibitory concentration (*mic*) was read as the lowest concentration of the substance that inhibited the development of visible bacterial growth after 24 hours of incubation. The minimal bactericidal concentration (*mbc*) was obtained by subculturing 0.1 ml from each negative tube and from the positive control tubes onto drug-free tryptose agar plates. The plates were incubated for 24 hours at 37°. The *mbc* is defined as the lowest concentration of substance from which subcultures were negative or which yielded fewer than three colonies. The antifungal screening was performed in a similar way. In this case, the compound solutions were prepared in Sabouraud dextrose broth (Difco), to contain *ca.* 10^5 cfu/ml of yeast and 100 mm^3 of inoculum was added to each test tube. The test tubes were incubated at 37° for 24-48 hours, and after this time were examined and the *mic* was recorded.

Proliferation inhibition Assay.

3T6 cells were seeded at a density of 160000 fibroblasts/well in a 24-well cluster on day 0. On day 3, the growth medium (dmem with 10% fetal bovine serum, Biokrom KG) was removed and the cells were fed with 1 ml of the growth medium containing various concentrations of the test compounds. After two days of incubation, on day 5, the cells were trypsinized and the cell number determined by counting in a Thoma chamber. The compound concentration at which the cell proliferation was inhibited to 50% of untreated control was taken as a measure of cytotoxicity.

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