Diels–Alder Reactions between Dienamides and Quinones: Stereochemistry of the Cycloadditions and Cytotoxic Activity of the Adducts

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Tetrahydronaphthoquinones and tetrahydroanthraquinones bearing an amido group have been prepared by Diels–Alder reactions between (*E*)-1-(*N*-carbobenzyloxyamino)-1,3-butadiene (2) or (*E*)-1-(*N*-benzoyl-*N*-benzylamino)-1,3-butadiene (5) and benzoquinone or 5-substituted naphthoquinones. The stereochemistry of the cycloadditions was investigated. A high regioselectivity was observed in the reaction of the diene carbamate 2 with 5-methoxy and 5-acetoxy naphthoquinones. This latter gave the unexpected 1,8-regioisomer 3d. The cycloadditions of the dienamide 5 with naphthoquinones 1 (R = OH, OMe, OAc) are regiospecific. Assignment of the structure of the tetrahydroanthraquinone 6b is in good agreement with the known directing effect of the 5-hydroxy group of juglone 1b in analogous Diels–Alder reactions. With 5-methoxy and 5-acetoxy naphthoquinones, the opposite regiochemistry observed is consistent with the electron-donating influence of the methoxy or acetoxy group, making the C-3 carbon atom more electron deficient. Aromatization of the adducts 6b and 7c was accompanied by an unusual elimination of the amido moiety. Thus, 1-hydroxy and 1-methoxy anthraquinones were obtained. Reactions of the dienes 2 and 5 with benzoquinone gave the tetrahydronaphthoquinones 9 and 10 with an *endo* stereospecificity. Oxidation of 9 by activated manganese dioxide gave the naphthoquinone 11. These compounds were submitted to *in vitro* cytotoxic assays towards murine L 1210 leukemia cells and clonogenic human tumor cell line MDA-MB 231. The naphthoquinone derivatives 9, 10 and 11 had significant activities with $IC_{50} \leqslant 0.4$ μg/ml towards these two tumor cell systems.

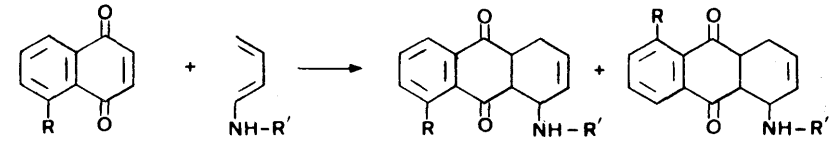
Keywords Diels–Alder reaction; dienamide; tetrahydroanthraquinone; tetrahydronaphthoquinone; cytotoxic activity

The Diels–Alder reaction between appropriate quinones and dienes offers an attractive route to functionalized naphthoquinones or anthraquinones of special interest in cancer chemotherapy. Several of these compounds exhibit antitumor activity,[1–5] but no examples of tetrahydro derivatives having this biological property have yet been described. Thus, we decided to examine some new tetrahydro amido anthraquinones and naphthoquinones specifically prepared by the [4+2] cycloaddition route.

Synthesis

In a preliminary publication[6] we reported on the Diels–Alder reaction between naphthoquinones **1** and (*E*)-1-(*N*-carbobenzyloxyamino)-1,3-butadiene **2**.[7]

A high regioselectivity was observed with **1c** and **1d** compared to **1b**. The last gave a mixture of the adducts **3b** and **4b** in a ratio of 3 : 1.[8] Identification of the regioisomers was made by aromatization of the adducts **3** and **4** followed by deprotection of the amino group and comparison with authentic samples of the corresponding aminoanthraquinones.[9] The inversion of the regiochemistry observed with the 5-methoxy naphthoquinone **1c** is consistent with the electron-donating influence of the 5-methoxy group making the C-3 carbon atom more electron-deficient.[10] With the 5-acetoxy naphthoquinone **1d**, the unexpected 1,8-regioisomer **3d** was regiospecifically obtained. This



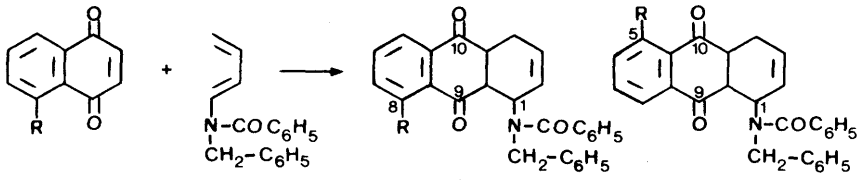
1 **2** **3** **4**

1a: R = H, R' = $COOCH_2C_6H_5$
1b: R = OH
1c: R = OMe
1d: R = OAc

3a: R = H
3b: R = OH
3c: R = OMe
3d: R = OAc

4b: R = OH
4c: R = OMe

Chart 1



1 **5** **6** **7**

1b: R = OH
1c: R = OMe
1d: R = OAc

6b: R = OH
7c: R = OMe
7d: R = OAc

Chart 2

anomaly is not unique in such cycloadditions and can be accomodated by considering secondary orbital interactions in the concerted transition state, as was pointed out by Alston *et al.*[11,12]

We describe, in the present work, the Diels–Alder reactions of (*E*)-1-(*N*-benzoyl-*N*-benzylamino)-1,3-butadiene[13] **5** and naphthoquinones **1** (R=OH, OMe, OAc) and investigate the regiochemistry of the cycloadditions.

The dienamide **5** and naphthoquinones **1** also underwent Diels–Alder reactions in benzene. The cycloadditions were regiospecific, as in each case only one isomer, **6b**, **7c** or **7d**, was obtained. Assignment of the structure of **6b** is in accord with the known directing effect of the 5-hydroxy group in **1b** in analogous Diels–Alder reactions with well polarized dienes when the cycloadditions were highly regioselective.[14] The opposite regiochemistry expected with **1c** and **1d** is corroborated by the infrared spectral data of these adducts (Table I).

It is apparent from Table I that the carbonyl frequencies in **7c** and **7d** are very close and this suggests similar structures. Thus, the values at 1705 cm^{-1} should be assigned to the hindered carbonyls at C-9 and those at 1680 and 1690 cm^{-1} to the normal carbonyls at C-10. This structural assignment is in good agreement with Kelly's hypothesis.[10]

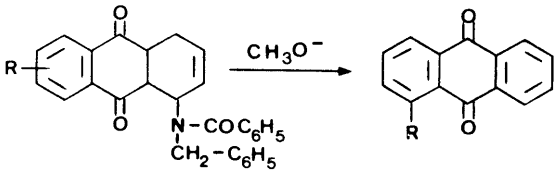
In contrast with the behavior of compounds **3** and **4**, aromatization of the adducts **6b** and **7c** was accompanied by the elimination of the amido group. Thus, we have obtained the mono substituted anthraquinones **8b**[15] and **8c**.[16]

Our attempts to avoid this unusual elimination by employing mild oxidizing agents such as activated manganese dioxide or dichlorodicyanobenzoquinone (DDQ) failed.

Reactions of the dienes **2** and **5** with benzoquinone proceeded similarly. Thus, the adducts **9** and **10** were obtained. The endo stereospecificity was evidenced by the proton nuclear magnetic resonance (^{1}H-NMR) spectra: the signal of H-10 appeared as a triplet with $J=5$ Hz. This value is in good agreement with a *cis* configuration for H-5, H-9 and H-10.[17] Aromatization of **9** by activated manganese dioxide gave **11**.

TABLE I.

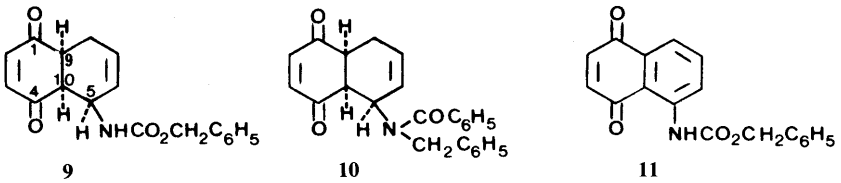
Compound	IR (KBr), ν cm^{-1} C=O (C-9)	C=O (C-10)	C=O (amide)
6b	1645 (chelated)	1690	1635
7c	1705	1680	1625
7d	1705	1690	1635



6b: R=−8OH
7c: R=−5OMe

8b: R=OH
8c: R=OMe

Chart 3



9 **10** **11**

Chart 4

Pharmacology

***In Vitro* Cytotoxicity towards L 1210 Leukemia Cells** The cytotoxic activity was evaluated towards L 1210 leukemia cells. Dose-effect relationships of the various compounds tested were determined from the regression line in a plot of percent cell growth inhibition against the logarithm of the dose. From these curves, the dose of drug reducing the cell growth by 50% after 48 h as compared to the control (IC_{50}) was estimated. Cytotoxicity data for the amido derivatives of tetrahydroanthraquinones and naphthoquinones are summarized in Table II.

***In Vitro* Cytotoxicity on Clonogenic Human Tumor Cell Line MDA-MB 231** The cytotoxic activity was evaluated towards a clonogenic human tumor cell line (MDA-MB 231) established from a human mammary adenocarcinoma.

TABLE II. Effect of the Amido Derivatives of Tetrahydroanthraquinones and Naphthoquinones on the Growth of L 1210 Cells

Compound	IC_{50} μg/ml	IC_{50} μM	Correlation equation
3a	6.668	18.47	$y=11.55x-39.19$
3c	5.227	13.36	$y=4.43x-11.48$
3d	5.736	13.69	$y=3.75x-9.12$
4c	8.45	21.26	$y=5.25x-15.64$
6b	4.228	9.65	$y=9.98x-31.19$
9	0.18	0.578	$y=3.61x-3.15$
10	0.194	0.522	$y=4.02x-4.21$
11	0.139	0.452	$y=3.93x-3.43$
Adriamycin	0.133	0.025	$y=3.05x+1.55$

TABLE III. Effect of the Amido Derivatives of Tetrahydroanthraquinones and Naphthoquinones on the Growth of MDA-MB 231 Cells

Compound	IC_{50} μg/ml	IC_{50} μM	Correlation equation
3a	8.15	22.59	$y=4.34x-12$
3d	14.64	34.94	$y=4.32x-12.99$
4c	12.31	31.48	$y=6.47x-21.47$
6b	16.24	37.07	$y=2.5x-5.52$
9	0.11	0.37	$y=4.38x-4.04$
10	0.40	1.08	$y=2.97x-2.74$
11	0.13	0.43	$y=3.6x-2.63$
Adriamycin	0.034	0.065	$y=2.23x+1.57$

The results are summarized in Table III.

Conclusion

We have prepared by the Diels–Alder route a series of tetrahydroanthraquinones and three naphthoquinone derivatives substituted with amido groups. These compounds were submitted to *in vitro* assays. The cytotoxicity towards L 1210 leukemia cells and MDA-MB 231 clonogenic human tumor cells was retained in some naphthoquinones derivatives, since significant activities with $IC_{50} \leqslant 0.4\ \mu g/ml$ were seen with compounds **9**, **10** and **11** towards the two tumor cell systems. These derivatives will be further examined *in vivo*.

Experimental

Melting points were measured on a Kofler apparatus. The infrared (IR) spectra (KBr discs) were recorded on a Perkin-Elmer 1310 spectrophotometer. The ^{1}H-NMR spectra were recorded at 80 MHz and 250 MHz on Bruker W.P. 80 and W.M. 250 apparatus. Chemical shifts are reported in ppm (δ) from tetramethylsilane (TMS) as an internal reference. The mass spectra (MS) were performed by direct ionization (EI at 70 eV) on an AE 1 MS 902 apparatus.

All solvents were dried and freshly distilled before use. The dienes **2**[7] and **5**[13] were prepared according to the cited literature. The cycloadditions were run under nitrogen.

1-(*N*-Carbobenzyloxyamino)-1,4,4a,9a-tetrahydro-9,10-anthraquinone (3a) The naphthoquinone **1a** (0.158 g, 1 mmol) and the diene carbamate **2** (0.203 g, 1 mmol) were dissolved in anhydrous toluene (5 ml). The solution was heated at 115 °C for 2 h. After evaporation of the solvent, the residue was precipitated by anhydrous Et_2O. Recrystallization from a mixture of ethyl acetate–hexane (4 : 6) gave **3a** as white needles (60% yield), mp 152 °C. IR (KBr): 3400 (ν NH), 1740 (ν CO ester), 1650 (ν CO ketone) cm^{-1}. ^{1}H-NMR (80 MHz : $CDCl_3$) δ: 8.1—7.66 (4H, m, H aromat.), 7.31 (5H, s, H aromat.), 6.15 (1H, br s, NH), 5.73 (2H, m, H_2 and H_3), 5.03 (2H, s, CH_2–C_6H_5), 4.63 (1H, m, H_1), 3.7—3.46 (2H, m, H_{9a} and H_{4a}), 2.34—2.15 (2H, m, H_4). MS *m/z*: 360 ($M^+ - 1$, 3), 358 (22), 210 (10), 208 (100), 152 (91), 108 (40), 91 (40).

1-(*N*-Carbobenzyloxyamino)-8-acetoxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (3d) Acetyljuglone (**1d** (0.216 g, 1 mmol) and the diene carbamate **2** (0.203 g, 1 mmol) were dissolved in anhydrous toluene (15 ml). The solution was heated at 115 °C for 90 min. After evaporation of the solvent, the residue was precipitated by anhydrous Et_2O. Recrystallization from anhydrous Et_2O gave **3d** as white needles (64% yield), mp 154 °C. IR (KBr): 3380 (ν NH), 1760 (ν CO ester), 1700 (ν CO ketone) cm^{-1}. ^{1}H-NMR (80 MHz: $CDCl_3$) δ: 8.06—7.25 (8H, m, H aromat.), 5.85 (1H, br s, NH), 5.7 (2H, m, H_2 and H_3), 5.07 (2H, s, CH_2–C_6H_5), 4.64 (1H, m, H_1), 3.67 (2H, m, H_{9a} and H_{4a}), 2.35 (3H, s, O–CO–CH_3), 2.16 (2H, m, H_4). MS *m/z*: 419 (M^+, 0.6), 418 ($M^+ - 1$, 2), 226 (58), 224 (26), 210 (100), 208 (9), 152 (20), 108 (20), 91 (70).

1-(*N*-Carbobenzyloxyamino)-5-methoxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (4c) Methyljuglone **1c** (0.188 g, 1 mmol) and the diene carbamate **2** (0.224 g, 1.2 mmol) were dissolved in anhydrous toluene (10 ml). The solution was heated at 115 °C for 5 h. The mixture was then cooled at room temperature: compound **4c** precipitated as a white solid, which was recrystallized from toluene (52% yield), mp 176 °C. IR (KBr): 3390 (ν NH), 1705, 1695, 1680 (ν CO) cm^{-1}. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 7.8—7.5 (3H, m, H aromat.), 7.3 (5H, s, H aromat.), 6.15 (1H, br s, NH), 5.72 (2H, m, H_2 and H_3), 5.05 (2H, s, CH_2–C_6H_5), 4.65 (1H, m, H_1) 3.95 (3H, s, OCH_3), 3.7—3.2 (2H, m, H_{9a} and H_{4a}), 2.45—2.15 (2H, m, H_4). MS *m/z*: 240 ($M^+ - NH_2$–$COOCH_2C_6H_5$, 100), 238 (22), 158 (12), 108 (77), 91 (90).

1-(*N*-Carbobenzyloxyamino)-8-methoxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (3c) The filtrate of **4c** contained a little of the 1,8-regioisomer **3c**. This compound was purified by column chromatography using a mixture of Et_2O–CH_2Cl_2 (1 : 9) as the eluant. The 1,8-regioisomer **3c** was eluted first (5% yield), mp 179—180 °C. IR (KBr): 3380 (ν NH), 1685 (ν CO br) cm^{-1}. ^{1}H-NMR (80 MHz: $CDCl_3$) δ: 7.8—7.2 (8H, m, H aromat.), 6.15 (1H, br s, NH), 5.75 (2H, m, H_2 and H_3), 5.05 (2H, s, CH_2–C_6H_5), 4.65 (1H, m, H_1), 3.95 (3H, s, OCH_3), 3.62 (1H, t, H_{9a}, $J = 5$ Hz), 3.45 (1H, m, H_{4a}), 2.35 (2H, m, H_4).

1-(*N*-Benzoyl-*N*-benzylamino)-8-hydroxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (6b) Juglone **1b** (0.392 g, 2.25 mmol) and the dienamide **5** (0.593 g, 2.25 mmol) were dissolved in benzene (5 ml) and the mixture was heated at 85 °C for 4 h. Evaporation of the solvent gave a residue, which was recrystallized from ethyl acetate to give **6b** (79% yield), mp 170 °C. IR (KBr): 3600—3100 (ν OH chelated), 1690, 1645 (ν CO ketone), 1635 (ν CO amide) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 12.65 (1H, s, peri-OH), 7.59—6.75 (13H, m, H aromat.), 5.62 (1H, br s, H_1), 5.42 (2H, m, H_2 and H_3), 4.5 (2H, s, CH_2–C_6H_5), 3.55 (1H, br s, H_{9a}), 2.75 (2H, m, H_{4a} and H_4), 1.63 (1H, m, H_4). MS *m/z*: 437 (M^+, 10), 226 ($M^+ - C_6H_5 - CH_2 - NH - CO - C_6H_5$, 20), 224 (20), 210 (16), 105 (13), 91 (6), 77 (11).

1-(*N*-Benzoyl-*N*-benzylamino)-5-methoxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (7c) Methyljuglone **1c** (0.188 g, 1 mmol) and the dienamide **5** (0.263 g, 1 mmol) were dissolved in freshly distilled benzene (5 ml). The solution was heated at 85 °C for 8 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate and precipitated by addition of hexane. Compound **7c** thus obtained was purified by crystallization from ethyl acetate–hexane (4 : 6). Yield (52%), mp 184 °C. IR (KBr): 1705, 1680 (ν CO ketone), 1625 (ν CO amide) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 7.74 (1H, m, H_7), 7.16—6.57 (12H, m, H aromat.), 5.56 (1H, m, H_1), 5.52 (1H, m, H_2), 5.30 (1H, m, H_3), 4.86 (2H, s, CH_2–C_6H_5), 4.16 (1H, m, H_{9a}), 3.35 (3H, s, OCH_3), 3.10 (1H, m, H_{4a}), 2.33 (1H, m, H_4), 1.75 (1H, m, H_4). MS (FAB positive) *m/z*: 452 ($M^+ + 1$, 71), 451 (M^+, 13), 241 (30), 212 (48), 210 (7), 154 (100).

1(*N*-Benzoyl-*N*-benzylamino)-5-acetoxy-1,4,4a,9a-tetrahydro-9,10-anthraquinone (7d) Acetyljuglone **1d** (0.216 g, 1 mmol) and the dienamide **5** (0.263 g, 1 mmol) were dissolved in freshly distilled benzene (5 ml), and the solution was heated at 85 °C for 24 h. After evaporation of the solvent, the residue was dissolved in anhydrous acetone and compound **7d** was precipitated by addition of an equal volume of anhydrous Et_2O. It was purified by crystallization from Et_2O, (54% yield), mp 180 °C. IR (KBr): 1705, 1690 (ν CO ketone), 1635 (ν CO amide) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 7.91 (1H, m, H_7), 7.20—6.66 (12H, m, H aromat.), 5.82 (1H, m, H_1), 5.47 (1H, m, H_2), 5.30 (1H, m, H_3), 4.74 (2H, s, CH_2–C_6H_5), 3.76 (1H, m, H_{9a}), 2.75 (1H, m, H_{4a}), 2.36 (1H, m, H_4), 2.15 (3H, s, $OCOCH_3$), 1.6 (1H, m, H_4). MS *m/z*: 224 ($M^+ - H_2$, $-H_2N - COOCH_2 - C_6H_5$, 91), 210 (40), 105 (100), 91 (10), 77 (40).

1-Hydroxy-9,10-anthraquinone (8b) The tetrahydroanthraquinone **6b** (0.437, 1 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (5 ml). Then, Na (0.023 g, 1 mmol) in 2 ml of CH_3OH was slowly added during 1 h. When the addition was complete, the yellow solution was neutralized with 1.0 N HCl to pH 7 and extracted with ether (2 × 10 ml). After evaporation of the solvent, the residue was recrystallized from ethanol to give **8b** (45% yield), mp 195 °C. (Lit.[15]: 194—195 °C).

1-Methoxy-9,10-anthraquinone (8c) Compound **8c** was prepared from **7c** as described above (50% yield), mp 172 °C (Lit.[16]: 169.5 °C).

5-(*N*-Carbobenzyloxyamino)-5,8,9,10-tetrahydro-1,4-naphthoquinone (9) Benzoquinone (0.266 g, 2.46 mmol) and the diene carbamate **2** (0.5 g, 2.46 mmol) were dissolved in benzene (15 ml). The solution was heated for 45 min at 85 °C and then the solvent was evaporated off. The residue was recrystallized from anhydrous ether to give **9** (78% yield), mp 136 °C. IR (KBr): 3380 (ν NH), 1685 (ν CO, br) cm^{-1}. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 7.36 (5H, s, H aromat.), 6.6 (2H, m, H_2 and H_3), 6.20 (1H, br s, NH), 5.72 (2H, m, H_6 and H_7), 5.10 (2H, s, CH_2–C_6H_5), 4.62 (1H, m, H_5), 3.5 (1H, t, H_{10}, $J = 5$ Hz), 3.44 (1H, m, H_9), 2.37—2.26 (2H, m, H_8). MS *m/z* 160 ($M^+ - NH_2COOCH_2C_6H_5$, 100), 158 (20), 108 (68), 91 (63), 77 (48).

5-(*N*-Benzoyl-*N*-benzylamino)-5,8,9,10-tetrahydro-1,4-naphthoquinone (10) Benzoquinone (0.411 g, 3.8 mmol) and the dienamide **5** (1 g, 3.8 mmol) were dissolved in CH_2Cl_2 (20 ml). The solution was heated at 55 °C for 2 h. After evaporation of the solvent, the residue was recrystallized from anhydrous Et_2O to give **10** (83% yield), mp 144 °C. IR (KBr): 1660 (ν CO ketone), 1630 (ν CO amide) cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 7.55—6.91 (10H, m, H aromat.), 6.75—6.66 (2H, dd, H_2 and H_3, $J = 4$ Hz), 5.78 (1H, m, H_6), 5.63 (1H, m, H_7), 5.32 (1H, m, H_5), 4.78 (2H, s, CH_2–C_6H_5), 4.16 (1H, t, H_{10}, $J = 5$ Hz), 3.38 (1H, m, H_9), 2.40 (2H, m, H_8). MS *m/z*: 371 (M^+, 4), 160 ($M^+ - C_6H_5CH_2 - NH - COC_6H_5$, 15), 158 (5), 105 (100), 91 (30), 77 (48).

5-(*N*-Carbobenzyloxyamino)-1,4-naphthoquinone (11) Compound **9** (0.153 g, 0.5 mmol) was dissolved in 9 ml of anhydrous choloroform. Then, activated manganese dioxide (0.216 g, 2.5 mmol) was added. The mixture was stirred for 20 min at room temperature and then filtered. The filtrate was evaporated to dryness. The residue was recrystallized from ethanol to give **11** as orange crystals (84% yield), mp 125 °C. IR (KBr): 3260 (ν NH), 1730, 1670, 1650 (ν CO) cm^{-1}. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 11.45 (1H, br s, NH), 8.84 (1H, dd, H_7, $J = 7$ Hz), 7.84—7.75 (2H, m, H_6 and H_8), 7.40 (5H, m, aromat.), 6.91 (2H, m, H_2 and H_3), 5.25 (2H, s, CH_2) MS *m/z*: 307 (M^+, 18), 91 (100), 77 (18).

Inhibitory Activity on L 1210 Cell Growth L 1210 murine leukemia cells were cultured in suspension in RPMI 1640 medium (EUROBIO) with 10% heat-inactivated fetal calf serum (Boehringer-Mannheim), 2-mercaptoethanol (10 μM), L-glutamine (2 mM) and antibiotics, at 37 °C, in a 5% CO_2 humidified air atmosphere.

Test compounds were dissolved in dimethylsulfoxide (DMSO). For the screening, the cell suspension (cells in an exponential growth phase) was adjusted to 5×10^4 or 5×10^5 viable cells per ml (cell viability was estimated by the Trypan-blue exclusion test). Cells were distributed in wells of a microtest tissue-culture plate (Falcon) before introducing the test compounds. Assays included solvent controls (final concentration of DMSO = 0.2%) and reference controls (Adriamycin). Four days later, cells were counted with a hemocytometer.

The percentage cell growth inhibition was calculated as follows:

$$\frac{T-t}{T} \times 100$$

T is the mean cell number in the control.

t is the mean cell number in the treated group.

IC_{50} is defined as the concentration inhibiting by 50% the cell growth compared to the control after 48 or 96 h of culture and is determined from the regression line of percentage cell growth inhibition as a function of the logarithm of the dose.

Inhibitory Activity on MDA-MB 231 Cell Growth MDA-MB 231 cells (line established from a human mammary adenocarcinoma) were a gift from Dr. F. Calvo, Hopital Saint-Louis, Paris. The cells were maintained in DMEM (GIBCO) supplemented with 10% fetal calf serum, insulin 1 UI/ml and antibiotics.

The effects of the drugs were assessed using a clonogenic assay.[18] A two-layer soft agar culture system was used as described by Salmon.[19] A total of 10^4 cells were plated in a volume of 1 ml (0.3% agar) over 2.5 ml base layers (0.5% agar) in 35 mm Petri dishes.

For drug assays, test compounds were incorporated into the top (cellular) layer at the time of plating. Malignant cells were incubated at 37 °C in a 5% CO_2 humidified air atmosphere and cell growth was evaluated in controls and treated assays 10 d after plating by counting colonies of more than 50 cells using an inverted microscope.

The micromolar concentration of each drug (IC_{50}) needed to reduce the growth of colonies to 50% of control values after seven days was determined for each compound from the regression line (percentage of colonies inhibition as a function of the dose) on logarithmic probability paper. Values of $IC_{50} < 4\ \mu$g/ml are considered as interesting cytotoxic activities.

References and Notes

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