Studies on Anthracenes. 3. Synthesis, Lipid Peroxidation and Cytotoxic Evaluation of 10-Substituted 1,5-Dichloro-9(10*H*)-anthracenone Derivatives

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The synthesis of a series of 1,5-dichloro-9(10*H*)-anthracenones bearing *O*-linked and *N*-linked substituents in the 10-position are described. Previous studies have shown that 9-acyloxy 1,5-dichloroanthracenes and 9-acyloxy 1,8-dichloroanthracenes displayed a potential cytotoxic effect. These results have encouraged us in further investigation of potential anthracenone derivatives. Therefore, a series of 10-substituted 1,8-dichloro-9(10*H*)-anthracenone derivatives were synthesized. These compounds were evaluated for their ability to inhibit the growth of human oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM 8401) and Chinese hamster ovary (CHO) cells, respectively. Compounds 3c and 4c of this series compare favorably in the KB cellular assay with mitoxantrone. Compound 4c showed combined inhibitory action against KB, GBM and CHO cell growth, respectively. In addition, redox property of the compounds for the inhibition of lipid peroxidation in model membranes was determined. Compounds 4b and 4d exhibited stronger antioxidant activity than ascorbic acid, (+)-α-tocopherol and mitoxantrone, respectively.

Key words anthracenone; anthracene; cytotoxic; lipid peroxidation; bromination; nucleophilic substitution

Anthracenone derivatives display potent and selective antitumor activity, but their mechanism of action is not yet clearly established. Despite structural similarities between the substituents, anthracenone nucleus, and molecules possessing known antitumor, antiproliferative, antipsoriatic, antiinflammatory, or antioxidant activity, these agents form a distinct mechanistic class.[1—3] Anthracene and anthracenone derivatives have been the subject of extensive research, mainly due to their well-recognized biological importance and significant biological applications. Although potential drug targets present only in cancerous cells have surfaced, the design of a drug which is selectively toxic to a tumor and not to the host organism is still very difficult.[4] We have previously shown that 9-acyloxy 1,5-dichloroanthracenes[5] and 9-acyloxy 1,8-dichloroanthracenes[6] provide useful templates for the design of potent antitumor derivatives. In previous papers, we described the synthesis, biological evaluation and structure–activity relationships for 9-acyloxy derivatives. In order to provide further insight into anthracene and the anthracenone pharmacophore, including the involvement of free radicals and antiproliferative activity, we examined the effects of introducing electron-donating 10-oxy and 10-N substituents to determine where replacement of the electron-withdrawing carbonyl of the earlier series can provide analogs with both potent antioxidant and antiproliferative activities. Despite the extensive and long-standing therapeutic utilization of anthracenones, their mechanism of action is still uncertain. A large body of evidence is consistent with a fundamental role of oxygen radicals in the induction of skin inflammation by anthracenes.[7] The mode of action of anthracenones leads to the conclusion that no single mechanism is predominantly operative, and oxygen radicals play a crucial role in the proinflammatory action. As noted above, cancer is typically characterized by a hyperproliferative component. There is thus a continuing need for effective compounds that address these aspects of cancer. To gain a wider understanding of the involvement of radicals in the action of anthracenone-derived agents, several related compounds bearing selected characteristic functional groups were designed. The approach was to develop structure–activity relationships (SARs) of 9(10*H*)-anthracenone analogs, with redox-active centers attached to the anthraquinone skeleton through spacer side chains at position 10, together with substituents with DNA-binding affinity. This paper describes the design and synthesis of anthracenones that incorporate in their structure a potential antioxidant component, as well as the results of relevant biologic studies.

Chemistry The introduction of side chains onto the anthracenone nucleus is usually accomplished by a stepwise procedure *via* the anthracenedione, because of the chemical instability of many anthracenones. Therefore, the reduction of 1,5-dichloroanthraquinone and bromination are required in the synthesis of C-10-substituted anthracenones. Although several excellent methods are available for the reduction of anthracenediones, many reducing systems do not lead directly to the anthracene stage.[8—10] The traditionally employed methods that lead preferentially to the anthracenones include stannous chloride in acetic acid/hydrochloric acid.[11,12] Thus, treatment of **5** with bromine provides **6**. Bromination of anthracenone takes place at the 10-position. The structure of compound **6** was confirmed by X-ray analysis. The ORTEP plot of **6** is shown in Fig. 2; the bond lengths and bond angles for this structure are listed in the experimental section. A series of 10-substituted 1,8-dichloro-9(10*H*)-anthracenones were synthesized from **6** by nucleophilic substitution at C-10 with 1.5—2.0 equivalents of appropriate amines or alcohols in the presence of catalytic amounts of $CaCO_3$, which strongly reduced the reaction time as compared to the noncatalyzed reaction. The 10-oxy-substituted and 10-N-substituted 1,8-dichloro-9(10*H*)-anthracenones

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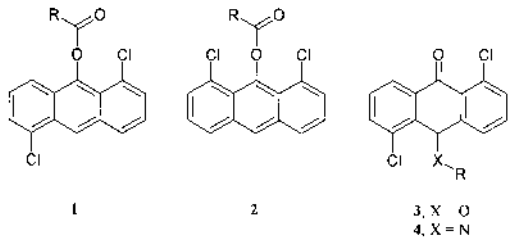


Fig. 1

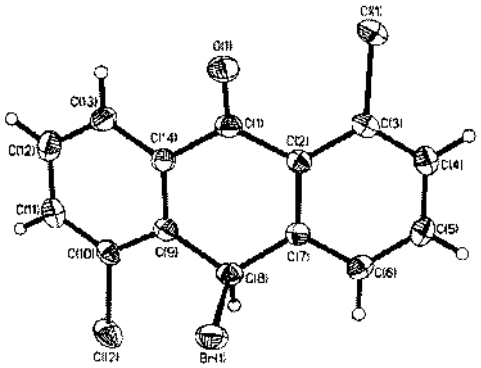
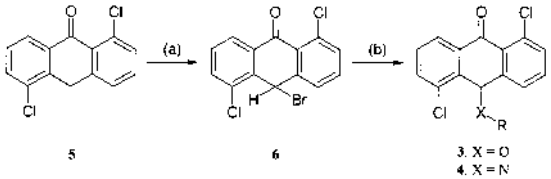


Fig. 2



(a): Br_2, CS_2; (b) $CaCO_3$, THF, N_2, R-OH or R-NH_2. R is defined in Table 1.

Chart 1

were readily synthesized according to Chart 1. The structures of these compounds were established on the basis of spectroscopic analysis. The ^{1}H-NMR spectra of **6** show a singlet at δ 6.62 for the C-10 of H, a doublet at δ 8.11 for the proton at position 8 and a dd at δ 7.65 for the proton at position 6. Of particular importance in these series compounds are the one proton chemical shifts at position 10 between δ 5.31 and 6.08, which are different from the range of the **5** possessing two 10-H protons at δ 4.32, and the IR stretching frequency, which is indicative of a C=O stretch. Furthermore, the ^{13}C-NMR spectra of these compounds show the usual carbonyl absorbance signal in the δ 180—190 region.[13,14]

Results and Discussion

In previous papers, we described the synthesis and some biological evaluation of 9-acyloxy 1,5-dichloroanthracenes and 9-acyloxy 1,8-dichloro anthracenes, respectively. In general, results from these assays did not show a reasonable correlation. We evaluated the ability of the compounds to inhibit the growth of human oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM8401) and Chinese hamster ovary (CHO) cells as normal cells, as well as lipid peroxidation in model membranes, respectively. The compounds were tested by a cytotoxic activity assay as demonstrated by the reduction in cell number

Table 1. *In Vitro* Cytotoxicity Activity of 10-Substituted 1,5-Dichloro-9(10*H*)-anthracenones

Compound	X–R	IC_{50} (μM)[a]		
		GBM[b]	KB[c]	CHO[d]
3a	OCH_3	23.5	8.8	2.9
3b	OCH_2CH_3	21.8	6.1	5.8
3c	$OCH_2CH_2CH_3$	11.1	1.8	42.0
3d	$OCH(CH_3)_2$	65.4	21.8	15.2
3e	$OCH_2CH_2CH_2CH_3$	29.5	29.5	8.0
3f	$OCH_2CH(CH_3)_2$	43.2	14.7	20.1
3g	$OCH_2C_6H_5$	18.5	93.4	24.7
4a	$N(CH_2CH_3)_2$	7.5	17.2	15.0
4b	$NH(C_6H_4)CH_3(m)$	11.2	9.5	6.2
4c	$NH(C_6H_4)CH_3(o)$	2.4	3.4	3.8
4d	$NH(C_6H_4)CH_3(p)$	4.6	11.3	3.0
Mitoxantrone–HCl		1.5	1.7	4.0

a) The cytotoxicity tests were replicated 2 times. Each treatment has 3 replications. IC_{50}, drug concentration inhibiting 50% of cellular growth following 48 h of drug exposure. *b*) Human cervical carcinoma cells of ME 180 (GBM8401). *c*) Human oral epidermoid carcinoma cells (KB cell line). *d*) Chinese hamster ovary (CHO) cells.

over time as compared to control plates. The results are shown in Table 1. Our study on the cytotoxicity evaluation of 10-substituted 1,8-dichloro-9(10*H*)-anthracenone derivatives revealed that compounds **4a**, **4c** and **4d** exhibited high cytotoxicity and significant activity on the GBM *in vitro* assay; compounds **3c**, **4b** and **4c** exhibited high cytotoxicity and significant activity on the KB *in vitro* assay. Only compounds **3a**, **4c** and **4d** were more toxic in CHO than mitoxantrone. Although there were no obvious requirements for potent antiproliferative activity, the inhibitory effects of these compounds appear to be due to some selective cell lines rather than to nonspecific redox properties. In addition to the redox properties, other factors such as an appropriate geometry of the molecules when bound to the active site of the substrate may be responsible for the cell growth inhibitory activities of the novel anthracenone and anthracene analogs.

The inhibitory effect on lipid peroxidation of these compounds was evaluated using rat brain phospholipid liposomes which provide an ideal model system for lipid peroxidation studies.[15] When compared to ascorbic acid, (+)-α-tocopherol and mitoxantrone, we found a better inhibitory effect at 0.5 mM by compounds **4b** and **4d** (Table 2). Furthermore, compounds **4b** and **4d** were significantly more efficient than ascorbic acid, (+)-α-tocopherol or mitoxantrone at 0.005 mM (Table 3). Although not a potent inhibitor of lipid peroxidation in itself, can provide a useful template for the design of potential anticancer agents. Moreover, the results support our hypothesis that structural modification of 1,5-dichloro-9(10*H*)-anthracenone may lead to control of the release of active oxygen species. In light of these findings, it is suggested that cytoyoxicity activity and lipid peroxidation alone is not sufficient for potent antiproliferative action. Whatever the molecular mechanism of the antiproliferative action of anthracenones, and wherever its locus, the results described herein indicate that it is sensitive to the slightest modification in the structure of anthracenone and that active analogs can be made only if the anthracenone moiety itself is retained. In conclusion, we have presented 10-substituted 1,8-dichloro-9(10*H*)-anthracenone derivatives which show potent inhibition of some selective cell lines. In order to understand

Table 2. Inhibitory Effect of 10-Substituted 1,5-Dichloro-9(10*H*)-anthracenones of the Invention on Iron-induced Lipid Peroxidation in Rat Brain Homogenates

Compound	X	R	Inhibition % (0.5 mM)[a]
3a	O	CH_3	7±0.1
3b	O	CH_2CH_3	7±0.1
3c	O	$CH_2CH_2CH_3$	29±2.5
3d	O	$CH(CH_3)_2$	13±1.3
3e	O	$CH_2CH_2CH_2CH_3$	31±3.7
3f	O	$CH_2CH(CH_3)_2$	38±3.1
3g	O	$CH_2C_6H_5$	16±1.5
4a	N	$(CH_2CH_3)_2$	12±1.4
4b	NH	$(C_6H_4)CH_3(m)$	100
4c	NH	$(C_6H_4)CH_3(o)$	73±3.5
4d	NH	$(C_6H_4)CH_3(p)$	100
Ascorbic acid			87±3.1
(+)-α-Tocopherol			53±4.4
Mitoxantrone–HCl			64±2.8

a) Relative percentage of inhibition. Inhibition was compared to that of the control [ascorbic acid, (+)-α-tocopherol and mitoxantrone–HCl], $p<0.01$, mean±S.E., $n=4$. Values are the mean percent inhibition at the indicated concentration (mM), and standard errors.

Table 3. Inhibitory Effects of **4b** and **4d** on Iron-Induced Lipid Peroxidation in Rat Brain Homogenates

Compound	Inhibition (%)[a]			
	5 mM	0.5 mM	0.05 mM	0.005 mM
4b	100	100	92±4.1	23±2.4
4d	100	100	94±3.5	33±2.7
Ascorbic acid	100	87±2.5	22±2.2	7±0.5
(+)-α-Tocopherol	100	53±1.7	0	0
Mitoxantrone–HCl	100	64±2.1	52±3.5	10±1.1

a) Relative percentage of inhibition. Inhibition was compared to that of the control [ascorbic acid, (+)-α-tocopherol and mitoxantrone–HCl], $p<0.01$, mean±S.E., $n=4$. Values are mean percent inhibition at the indicated concentration (mM), and standard errors.

whether these compounds have potent antitumor and biological activities, we will examine their effects in other tests and the results will be reported elsewhere.

Experimental

All temperatures are reported in degrees centigrade. Melting points were determined with a Büchi B-545 melting point apparatus and are uncorrected. Chromatography refers to column chromatography using silica gel (E. Merck, 70—230 mesh). ^{1}H-NMR spectra were recorded with a Varian GEMR-H-300 (300 MHz); δ values are in ppm relative to a tetramethylsilane internal standard. Fourier-transform IR spectra (KBr) were recorded on a Perkin-Elmer 983G spectrometer. Mass spectra (EI, 70 eV, unless otherwise stated) were obtained on a Finnigan MAT TSQ-46 and Finnigan MAT TSQ-700. UV spectra were recorded on a Shimadzu UV-160.

10-Bromo 1,5-Dichloro-9(10*H*)-anthracenone (6)[11] To a solution of 1,5-dichloro-9(10*H*)-anthracenone[5] (16.0 mmol) in CS_2 (30 ml) was added dropwise a solution of bromine (20.0 mmol) in CS_2 (5 ml). The reaction mixture was refluxed for 1 h. The reaction mixture was allowed to cool, filtered, and the filtrate was evaporated to dryness. The remaining crude product was dissolved in dichloromethane. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solution was concentrated. The resulting precipitate was collected by filtration, and further purified by crystallization and chromatography to give the corresponding product: 95% yield. mp 201—202 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.11 (1H, d, J=7.1 Hz), 7.65 (1H, dd, J=1.2, 8.0 Hz), 7.61—7.46 (4H, m), 6.62 (1H, s). MS *m/z*: 341 (M^+), 261. UV λ_{max} ($CHCl_3$) nm (log ε): 295 (4.57). IR (KBr) cm^{-1}: 1670.

X-Ray Crystal Structure of **6**: $C_{14}H_7BrCl_2O$, Mt=342.01. A needle of the approximate dimensions 0.5×0.4×0.12 mm was mounted to glass fiber along its longest axis. The crystal system was triclinic, space group *P*-1. Cell constants a=7.6092(13), b=8.5639(14), and c=10.0242(14) Å; α=76.212(11)°; β=73.058(12)°; γ=87.135(12)°; V=606.7(2) Å^3; Z=2; μ(Mo$K\alpha$)=3.809 cm^{-1}; F(000)=336. Cell dimensions were determined using a Nonious CAD4 Kappa Axis XRD & Siemens Smart CCD XRD diffractometer equipped with a graphite monochromator and molybdenum source (λ=7.1073 Å). Data were collected on the same instrument using ω scans with 2θ varying from 2—50°. A total of 3090 unique reflections were determined, of which 1647 were >2.0σ. The structure was solved using direct methods and was refined using standard techniques. A total of 235 parameters were varied in the final least-squares. The refinement converged at R=0.040 and R_w=0.040. Residual electron density varied from 0.20 to −0.20 e/Å^3.

General Procedure for the Preparation of 10-Substituted 1,5-Dichloro-9(10*H*)-anthracenones To a solution of **6** (2.0 mmol) and anhydrous calcium carbonate (0.5 g) in dry THF (20 ml) was added dropwise a solution of an appropriate alcohol or amine (3 mmol) in dry THF (10 ml) under N_2. The reaction mixture was stirred at room temperature or refluxed for several hours. Water (250 ml) was added and then extracted with dichloromethane. The combined organic extracts were washed with water, dried ($MgSO_4$), and concentrated. The resulting precipitate was collected by filtration, washed with water and further purified by crystallization and chromatography.

10-Methoxy 1,5-Dichloro-9(10*H*)-anthracenone (**3a**): 90% yield. mp 170—171 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, dd, J=0.9, 7.6 Hz), 7.63 (1H, dd, J=1.0, 7.4 Hz), 7.55 (1H, dd, J=2.3, 6.6 Hz), 7.52 (1H, d, J=2.5 Hz), 7.51 (1H, t, J=7.3 Hz), 7.45 (1H, t, J=7.8 Hz), 5.76 (1H, s), 3.1 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 183.55, 141.99, 137.13, 135.76, 135.37, 134.94, 134.39, 133.60, 133.36, 130.65, 130.18, 129.28, 126.76, 72.52, 54.67. MS *m/z*: 292 (M^+), 261. IR (KBr) cm^{-1}: 1670, 1068. UV λ_{max} ($CHCl_3$) nm (log ε): 282 (4.72). *Anal.* Calcd for $C_{15}H_{10}O_2Cl_2$: C, 49.15; H, 3.44. Found: C, 49.41; H, 3.21.

10-Ethoxy 1,5-Dichloro-9(10*H*)-anthracenone (**3b**): 95% yield. mp 153—154 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, dd, J=0.8, 7.6 Hz), 7.61 (1H, dd, J=0.8, 7.6 Hz), 7.54 (1H, dd, J=2.1, 6.6 Hz), 7.51(1H, d, J=5.7 Hz), 7.49 (1H, t, J=7.7 Hz), 7.43 (1H, t, J=7.8 Hz), 5.79 (1H, s), 3.32—3.25 (2H, m), 1.04 (3H, t, J=6.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 183.67, 142.67, 137.06, 136.35, 135.26, 134.85, 134.31, 133.52, 133.16, 130.47, 130.07, 129.17, 126.71, 71.66, 62.87, 15.76. MS *m/z*: 306 (M^+), 261. IR (KBr) cm^{-1}: 1678, 1069. UV λ_{max} ($CHCl_3$) nm (log ε): 280 (4.88). *Anal.* Calcd for $C_{16}H_{12}O_2Cl_2$: C, 62.54; H, 3.94. Found: C, 62.18; H, 3.85.

10-Propyloxy 1,5-Dichloro-9(10*H*)-anthracenone (**3c**): 62% yield. mp 98—99 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, dd, J=0.8, 7.6 Hz), 7.62 (1H, dd, J=0.9, 7.6 Hz), 7.55 (1H, dd, J=2.2, 6.5 Hz), 7.52—7.48 (2H, m), 7.44 (1H, t, J=7.7, 7.9 Hz), 5.82 (1H, s), 3.14 (2H, t, J=6.4 Hz), 1.44—1.37 (2H, m), 0.73 (3H, t, J=7.2, 7.4 Hz). ^{13}C-NMR ($CDCl_3$) δ: 183.65, 142.74, 137.08, 136.33, 135.22, 134.92, 134.35, 133.54, 133.14, 130.46, 130.09, 129.23, 71.60, 68.75, 23.45, 11.07. MS *m/z*: 320 (M^+), 261. IR (KBr) cm^{-1}: 1678, 1051. UV λ_{max} ($CHCl_3$) nm (log ε): 281 (4.66). *Anal.* Calcd for $C_{17}H_{14}O_2Cl_2$: C, 63.55; H, 4.39. Found: C, 63.28; H, 4.23.

10-(2-Propyloxy) 1,5-Dichloro-9(10*H*)-anthracenone (**3d**): 58% yield. mp 172—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.99 (1H, dd, J=0.9, 7.6 Hz), 7.58 (1H, dd, J=1.0, 7.6 Hz), 7.50 (1H, dd, J=2.6, 7.0 Hz), 7.48—7.44 (2H, m), 7.41 (1H, t, J=7.8 Hz), 5.83 (1H, s), 3.6 (1H, m), 1.06—0.88 (6H, dd, J=6.0, 6.1 Hz). ^{13}C-NMR ($CDCl_3$) δ: 84.19, 143.29, 137.43, 137.22, 134.19, 134.42, 134.06, 133.23, 133.02, 130.39, 129.05, 126.84, 69.64, 68.81, 23.61, 22.80. MS *m/z*: 320 (M^+), 261. IR (KBr) cm^{-1}: 1679, 1016. UV λ_{max} ($CHCl_3$) nm (log ε): 287 (4.77). *Anal.* Calcd for $C_{17}H_{14}O_2Cl_2$: C, 62.54; H, 3.94. Found: C, 62.32; H, 3.73.

10-(Butyloxy) 1,5-Dichloro-9(10*H*)-anthracenone (**3e**): 50% yield. mp 117—118 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, dd, J=0.9, 7.7 Hz), 7.61 (1H, dd, J=1.0, 7.7 Hz), 7.55 (1H, dd, J=2.2, 6.5 Hz), 7.52—7.48 (2H, m), 7.44 (1H, t, J=7.8 Hz), 5.81 (1H, s), 3.18 (2H, t, J=6.3 Hz), 1.36 (2H, m), 1.17 (2H, m), 0.72 (3H, t, J=7.4 Hz). ^{13}C-NMR ($CDCl_3$) δ: 183.66, 142.78, 137.12, 136.34, 135.24, 134.91, 134.33, 133.50, 133.13, 130.44, 130.13, 129.20, 126.67, 71.58, 66.63, 32.24, 19.66, 14.14. MS *m/z*: 334 (M^+), 261. IR (KBr) cm^{-1}: 1678, 1060. UV λ_{max} ($CHCl_3$) nm (log ε): 280 (4.84). *Anal.* Calcd for $C_{18}H_{16}O_2Cl_2$: C, 64.48; H, 4.81. Found: C, 64.21; H, 4.89.

10-(*iso*-Butyloxy) 1,5-Dichloro-9(10*H*)-anthracenone (**3f**): 65% yield. mp 146—147 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, dd, J=0.8, 7.9 Hz), 7.62 (1H, dd, J=0.9, 7.6 Hz), 7.55 (1H, dd, J=2.4, 6.3 Hz), 7.53 (1H, d, J=6.1 Hz), 7.50 (1H, t, J=6.5 Hz), 7.44 (1H, t, J=7.8 Hz), 5.83 (1H, s), 2.92—2.87 (2H, m), 1.66—1.54 (1H, m), 0.70 (6H, t, J=6.8, 6.7 Hz). ^{13}C-NMR ($CDCl_3$) δ: 183.62, 142.78, 137.07, 136.29, 135.18, 134.95, 134.36, 133.54, 133.11, 130.43, 130.09, 129.26, 126.61, 73.50, 71.52, 29.03, 19.84, 19.79.

MS *m/z*: 334 (M^+), 261. IR (KBr) cm^{-1}: 1681, 1056. UV λ_{max} ($CHCl_3$) nm (log ε): 281 (4.55). *Anal.* Calcd for $C_{18}H_{16}O_2Cl_2$: C, 64.48; H, 4.81. Found: C, 64.23; H, 4.75.

10-Benzyloxy 1,5-Dichloro-9(10*H*)-anthracenone (**3g**): 75% yield. mp 150—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.05 (1H, d, J=7.6 Hz), 7.62 (1H, d, J=8.0 Hz), 7.57 (1H, d, J=6.9 Hz), 7.55—7.51 (2H, m), 7.45 (1H, t, J=7.8 Hz), 7.23—7.14 (5H, m), 5.96 (1H, s), 4.27 (2H, dd, J=6.5, 10.9 Hz). ^{13}C-NMR ($CDCl_3$) δ: 183.56, 142.32, 137.96, 137.21, 136.09, 135.41, 134.91, 134.43, 133.64, 133.38, 130.69, 130.20, 129.38, 128.85, 128.43, 128.34, 126.83, 71.57, 69.29. MS *m/z*: 368 (M^+), 261. IR (KBr) cm^{-1}: 1676, 1047. UV λ_{max} ($CHCl_3$) nm (log ε): 280 (4.83). *Anal.* Calcd for $C_{21}H_{14}O_2Cl_2$: C, 68.29; H, 3.82. Found: C, 68.18; H, 3.68.

10-Diethylamino 1,5-Dichloro-9(10*H*)-anthracenone (**4a**): 64% yield. mp 192—193 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, dd, J=1.9, 7.7 Hz), 7.59 (1H, dd, J=1.0, 7.8 Hz), 7.47—7.31 (4H, m), 5.31 (1H, s), 2.59—2.20 (4H, m), 0.92 (6H, t, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 184.67, 142.83, 138.91, 138.00, 134.96, 134.25, 132.71, 131.96, 130.79, 129.30, 128.56, 125.91 58.04, 44.06, 14.13. MS *m/z*: 333 (M^+), 261. IR (KBr) cm^{-1}: 1674, 1299. UV λ_{max} ($CHCl_3$) nm (log ε): 275 (4.97). *Anal.* Calcd for $C_{18}H_{17}NOCl_2$: C, 64.67; H, 5.12. Found: C, 64.48; H, 5.35.

10-(*m*-Toluidino) 1,5-Dichloro-9(10*H*)-anthracenone (**4b**): 66% yield. mp 186—188 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.08 (1H, dd, J=1.0, 7.7 Hz), 7.65 (1H, dd, J=1.2, 7.8 Hz), 7.50—7.33 (4H, m), 7.07 (1H, t, J=7.8 Hz), 6.61 (1H, s), 6.63 (1H, s), 6.56 (1H, s), 6.05 (1H, s), 3.72 (1H, s), 2.26 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 183.56, 146.28, 145.88, 139.73, 138.20, 136.22, 135.35, 134.71, 134.55, 133.92, 132.50, 129.87, 129.76, 128.41, 127.54, 126.85, 121.14, 117.38, 113.30, 52.63, 22.12. MS *m/z*: 367 (M^+), 261. IR (KBr) cm^{-1}: 3359, 1664. UV λ_{max} ($CHCl_3$) nm (log ε): 272 (5.17). *Anal.* Calcd for $C_{21}H_{15}NOCl_2$: C, 68.48; H, 4.10. Found: C, 68.27; H, 4.35.

10-(*o*-Toluidino) 1,5-Dichloro-9(10*H*)-anthracenone (**4c**): 58% yield. mp 193—195 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, dd, J=0.8, 8.4 Hz), 7.66 (1H, dd, J=1.3, 7.9 Hz), 7.50—7.26 (4H, m), 7.16 (1H, d, J=4.1 Hz), 7.01 (1H, d, J=7.4 Hz), 6.77—6.72 (2H, m), 6.08 (1H, s), 3.68 (1H, s), 1.87 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 183.56, 146.02, 144.37, 138.31, 136.31, 135.39, 134.79, 134.63, 133.89, 132.52, 131.30, 129.96, 127.62, 127.34, 126.94, 125.21, 119.97, 114.76, 114.72, 52.89, 17.98. MS *m/z*: 367 (M^+), 261. IR (KBr) cm^{-1}: 3402, 1658. UV λ_{max} ($CHCl_3$) nm (log ε): 276 (5.24). *Anal.* Calcd for $C_{21}H_{15}NOCl_2$: C, 68.48; H, 4.10. Found: C, 68.19; H, 4.26.

10-(*p*-Toluidino) 1,5-Dichloro-9(10*H*)-anthracenone (**4d**): 73% yield. mp 189—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.06 (1H, dd, J=1.0, 7.8 Hz), 7.65 (1H, dd, J=1.2, 7.9 Hz), 7.46—7.40 (3H, m), 7.35 (1H, t, J=7.2 Hz), 6.97 (2H, d, J=8.0 Hz), 6.65 (2H, d, J=8.3 Hz), 5.97 (1H, s), 3.69 (1H, s), 2.23 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 183.54, 145.79, 143.76, 138.25, 136.24, 135.33, 134.69, 134.47, 133.82, 132.46, 130.43, 129.92, 129.84, 128.47, 127.66, 126.85, 117.24, 53.44, 21.07. MS *m/z*: 367 (M^+), 261. IR (KBr) cm^{-1}: 3381, 1658. UV λ_{max} ($CHCl_3$) nm (log ε): 275 (5.09). *Anal.* Calcd for $C_{21}H_{15}NOCl_2$: C, 68.48; H, 4.10. Found: C, 68.56; H, 4.23.

Cytotoxic Activity Test Human oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM8401) and Chinese hamster ovary (CHO) cells grown in plateau phase were cultivated, and the cell proliferation assay was performed as previously described.[6] Inhibition of cellular growth was calculated by comparison of the mean values of the test compound (n=3) with the control (n=6—8) activity: (1−test compound/control)×100.

Assay of Lipid Peroxidation Rat brain homogenate was prepared from the brains of freshly killed Wistar rats, and its peroxidation in the presence of iron ions was measured by the thiobarbituric acid (TBA) method as previously described.[5,15] The extent of lipid peroxidation was estimated as thiobarbituric acid-reactive substances and was read at 532 nm in a spectrophotometer (Shimadzu UV-160). The results of this assay are provided in Tables 2 and 3.

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